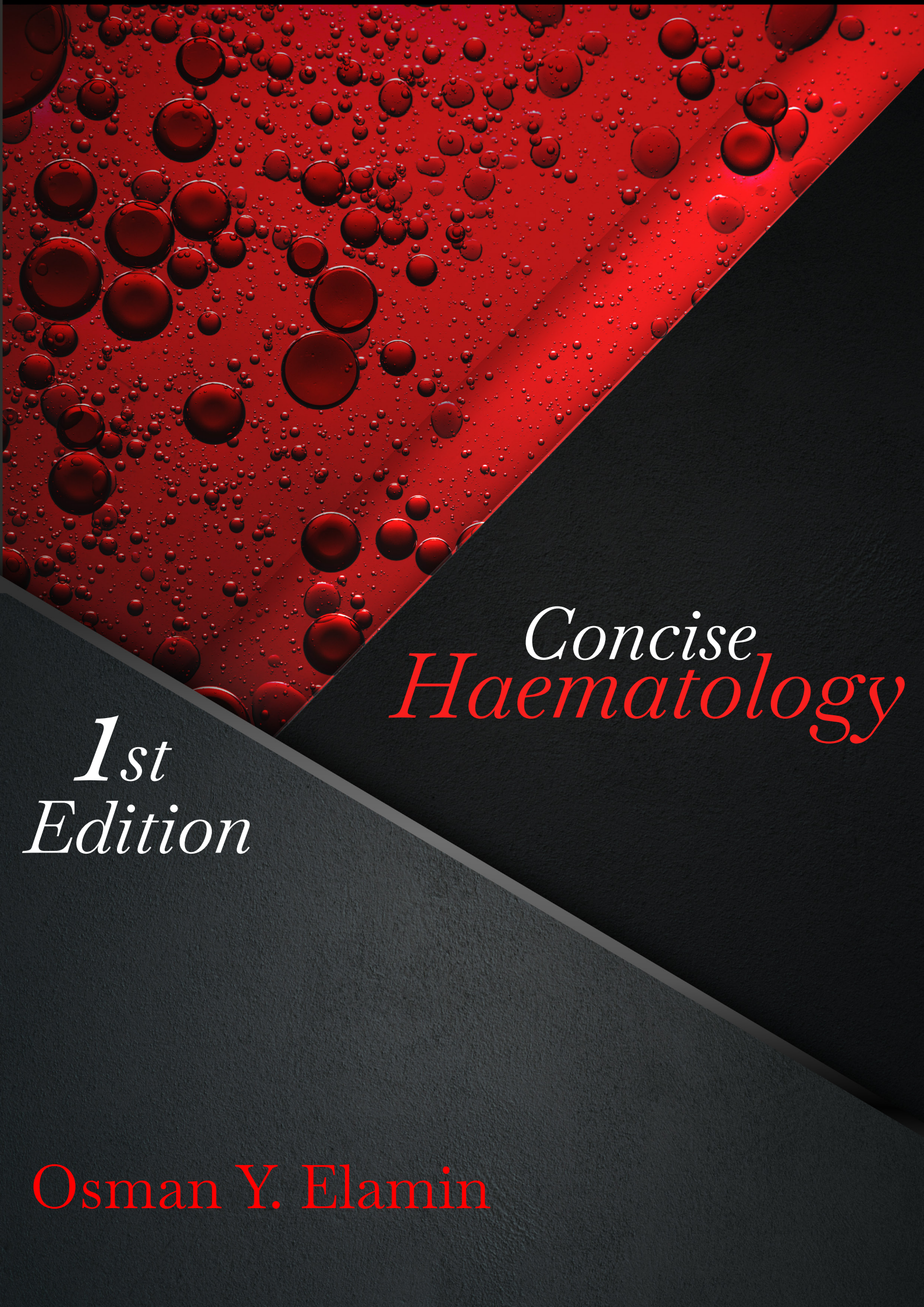
Concise
Haematology
1st
Edition
Osman Y. Elamin

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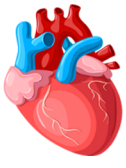
Designed with  *By: Hamad Y.Aljack*

Concise Haematology Book

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PREFACE

Haematology is a large field of medicine, it is very complicated and has many branches. You can find that it's too hard to summarize all haematology in a small book, so in this book we tried to take the majority of topics that belong to Haematology to make it easier to study by medical students.

In this book we defined haematology, blood and blood forming organs also the physiological, biochemical and immunological processes involved normal blood formation and function, coagulation and normal hemostasis. Finally we mention the blood disorders and the disturbances that may occur during blood formation.

We hope the present book will enable the medical student to grasp the essential features of modern clinical and laboratory haematology.

We would like to thank our colleagues and assistants who have helped with the preparation of the book. In particular Rehab Omer Adam (Assotiated Professor, Elimam Almahadi university), Izzaldin Ahmed Osman
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Haematology Introduction

A master of blood once said "Do you not think that there are things that which you cannot understand, and yet which are; that some people see things that others cannot? But there are things old and new which must not be contemplate by men ' s eyes, because they know –or think they know- some things which other men have told them.

Ah, it is the fault of our science that it wants to explain all; and if it explain not, then it says there is nothing to explain. " *Dracula . (B. Stoker, Dracula P205, 1986)*

And we are here to prove him wrong and try to explain it all , We are the people who see things that others cannot.

Haematology Introduction

Haematology, also spelled hematology, is the branch of medicine concerned with the study of the cause, prognosis, treatment, and prevention of diseases related to blood. It involves treating diseases that affect the production of blood and its components, such as blood cells, hemoglobin, blood proteins, bone marrow, platelets, blood vessels, spleen, and the mechanism of coagulation(1)Such diseases might include hemophilia, blood clots, other bleeding disorders and blood cancers such as leukemia, multiple myeloma, and lymphoma. The laboratory work that goes into the study of blood is frequently performed by a medical technologist or medical laboratory scientist. Many hematologists work as hematologist-oncologists, also providing medical treatment for all types of cancer.

Blood

Definition: Blood is a body fluid in humans and other animals that delivers necessary substances such as nutrients and oxygen to the cells and transports metabolic waste products away from those same cell(2). Blood is considered a specialized form of connective tissue, given its origin in the bones and the presence of potential molecular fibers in the form of fibrinogen.

In vertebrates, blood is composed of blood cells suspended in blood plasma. Plasma, which constitutes 55% of blood fluid, is mostly water (92% by volume), and contains proteins, glucose, mineral ions, hormones, carbon dioxide (plasma being the main medium for excretory product transportation), and blood cells themselves. Albumin is the main protein in plasma, and it functions to regulate the colloidal osmotic pressure of blood. The blood cells are mainly red blood cells (also called RBCs or erythrocytes), white blood cells (also called WBCs or leukocytes) and platelets (Plts also called thrombocytes). The most abundant cells in vertebrate blood are red blood cells. These contain hemoglobin, an iron-containing protein, which facilitates oxygen transport by reversibly binding to this respiratory gas and greatly increasing its solubility in blood. In contrast, carbon dioxide is mostly transported extracellular-

ly as bicarbonate ion transported in plasma. Vertebrate blood is bright red when its hemoglobin is oxygenated and dark red when it is deoxygenated. Some animals, such as crustaceans and mollusks, use hemocyanin to carry oxygen, instead of hemoglobin. Insects and some mollusks use a fluid called hemolymph instead of blood, the difference being that hemolymph is not contained in a closed circulatory system. In most insects, this "blood" does not contain oxygen-carrying molecules such as hemoglobin because their bodies are small enough for their tracheal system to suffice for supplying oxygen. Jawed vertebrates have an adaptive immune system, based largely on white blood cells. White blood cells help to resist infections and parasites. Platelets are important in the clotting of blood.

Blood is circulated around the body through blood vessels by the pumping action of the heart. In animals with lungs, arterial blood carries oxygen from inhaled air to the tissues of the body, and venous blood carries carbon dioxide, a waste product of metabolism produced by cells, from the tissues to the lungs to be exhaled.

Functions of blood

Blood performs many important functions within the body, including:

a) Supply of oxygen to tissues (bound to hemoglobin, which is carried in red cells).

b) Supply of nutrients such as glucose, amino acids, and fatty acids (dissolved in the blood or bound to plasma proteins (e.g., blood lipids)).

c) Removal of waste such as carbon dioxide, urea, and lactic acid.

d) Immunological functions, including circulation of white blood cells, and detection of foreign material by antibodies.

e) Coagulation, the response to a broken blood vessel, the conversion of blood from a liquid to a semisolid gel to stop bleeding.

f) Messenger functions, including the transport of hormones and the signaling of tissue damage.

g) Regulation of core body temperature.

h) Hydraulic functions.

Blood Constituents

In mammals Blood accounts for 7% of the human body weight(3) with an average density around 1060 kg/m^3 very close to pure water's density of 1000 kg/m^3. The average adult has a blood volume of roughly 5 liters, which is composed of plasma and several kinds of cells. These blood cells (which are also called corpuscles or "formed elements") consist of erythrocytes (red blood cells, RBCs), leukocytes (white blood cells) and it has two main groups' myelocytes (monocytes, eosinophils, basophils, and neutrophils) and lymphocytes. And thrombocytes (platelets). By volume, the red blood cells constitute about 45% of whole blood, the plasma about 54.3%, and white cells

about 0.7%.

Whole blood (plasma and cells) exhibits non-Newtonian fluid dynamics. If all human hemoglobin were free in the plasma rather than being contained in RBCs, the circulatory fluid would be too viscous for the cardiovascular system to function effectively.

Human blood fractioned by centrifugation: Plasma (upper, yellow layer), buffy coat (middle, thin white layer) and erythrocyte layer (bottom, red layer).

One microliter of blood contains: 4.7- 6.1 million (male), 4.2 to 5.4 million (female) erythrocytes (Red blood cells), 4,000–11,000 leukocytes (White blood cells), and 200,000–500,000 thrombocytes (platelets)(4).

Constitution of normal blood Parameter Value: Hematocrit 45 ± 7 (38–52%) for males 42 ± 5 (37–47%) for females, pH 7.35–7.45, base excess −3 to +3, PO2 10–13 kPa (80–100 mm Hg), PCO2 4.8–5.8 kPa (35–45 mm Hg), HCO3−21–27 mm, Oxygen saturation (Oxygenated: 98–99% Deoxygenated: 75%).

Haemoglobin

Hemoglobin (American) or haemoglobin (British); abbreviated Hb or Hgb, is the iron-containing oxygen-transport metalloprotein in the red blood cells (erythrocytes) of almost all vertebrates (the exception being the fish family Channichthyidae) as well as the tissues of some invertebrates.

Haemoglobin in the blood carries oxygen from the lungs or gills to the rest of the body (i.e. the tissues). There it releases the oxygen to permit aerobic respiration to provide energy to power the functions of the organism in the process called metabolism. A healthy individual has 12 to 16 grams of haemoglobin in every 100 ml of blood.

Hemoglobin types in human:

In the embryo: Gower 1 (ζ2ε2), Gower 2 (α2ε2), Hemoglobin Portland I (ζ2γ2), Hemoglobin Portland II (ζ2β2).

In the fetus: Hemoglobin F (α2γ2).

After birth: Hemoglobin A (α2β2) (Chr. 16 p13.3), the most common with a normal amount over 95%.

Hemoglobin A2 (α2δ2) (Chr. 16 p13.3) δ chain synthesis begins late in the third trimester and, in adults, it has a normal range of 1.5–3.5%.

Hemoglobin F (α2γ2) – In adults Hemoglobin F is restricted to a limited population of red cells called F-cells. However, the level of Hb F can be elevated in persons with sickle-cell disease and beta-thalassemia.

In mammals, the protein makes up about 96% of the red blood cells' dry content (by weight), and around 35% of the total content (including water).Haemoglobin has an oxygen-binding capacity of 1.34 mL O2 per gram, which increases the total blood oxygen capacity seventy-fold compared to dissolved oxygen in blood. The mammalian

hemoglobin molecule can bind (carry) up to four oxygen molecules. Hemoglobin is involved in the transport of other gases: It carries some of the body's respiratory carbon dioxide (about 20–25% of the total) as carbaminohemoglobin, in which CO2 is bound to the heme protein. The molecule also carries the important regulatory molecule nitric oxide bound to a globin protein thiol group, releasing it at the same time as oxygen.

Haemoglobin is also found outside red blood cells and their progenitor lines. Other cells that contain haemoglobin include the A9 dopaminergic neurons in the substantia nigra, macrophages, alveolar cells, lungs, retinal pigment epithelium, hepatocytes, mesangial cells in the kidney, endometrial cells, cervical cells and vaginal epithelial cells. In these tissues, haemoglobin has a non-oxygen-carrying function as an antioxidant and a regulator of iron metabolism.

Haemoglobin Synthesis

Hemoglobin (Hb) is synthesized in a complex series of steps. The heme part is synthesized in a series of steps in the mitochondria and the cytosol of immature red blood cells, while the globin protein parts are synthesized by ribosomes in the cytosol (5) Production of Hb continues in the cell throughout its early development from the proerythroblast to the reticulocyte in the bone marrow. At this point, the nucleus is lost in mammalian red blood cells, but not in birds and many other species. Even after the loss of the nucleus in mammals, residual ribosomal RNA allows further synthesis of Hb until the reticulocyte loses its RNA soon after entering the vasculature (this hemoglobin-synthetic RNA in fact gives the reticulocyte its reticulated appearance and name)(6)

Hemoglobin Structure

Hemoglobin has a quaternary structure characteristic of many multi-subunit globular proteins (7) Most of the amino acids in hemoglobin form alpha helices, and these helices are connected by short non-helical segments. Hydrogen bonds stabilize the helical sections inside this protein, causing attractions within the molecule, which then causes each polypeptide chain to fold into a specific shape. Hemoglobin's quaternary structure comes from its four subunits in roughly a tetrahedral arrangement.

In most vertebrates, the hemoglobin molecule is an assembly of four globular protein subunits. Each subunit is composed of a protein chain tightly associated with a non-protein prosthetic heme group. Each protein chain arranges into a set of alpha-helix structural segments connected together in a globin fold arrangement. Such a name is given because this arrangement is the same folding motif used in other heme/globin proteins such as myoglobin (8) this folding pattern contains a pocket that strongly binds the heme group.

A heme group consists of an iron (Fe) ion (charged atom) held in a heterocyclic ring,

known as a porphyrin. This porphyrin ring consists of four pyrrole molecules cyclically linked together (by methine bridges) with the iron ion bound in the center. The iron ion, which is the site of oxygen binding, coordinates with the four nitrogen atoms in the center of the ring, which all lie in one plane. The iron is bound strongly (covalently) to the globular protein via the N atoms of the imidazole ring of F8 histidine residue (also known as the proximal histidine) below the porphyrin ring. A sixth position can reversibly bind oxygen by a coordinate covalent bond, completing the octahedral group of six ligands. Oxygen binds in an "end-on bent" geometry where one oxygen atom binds to Fe and the other protrudes at an angle. When oxygen is not bound, a very weakly bonded water molecule fills the site, forming a distorted octahedron. Even though carbon dioxide is carried by hemoglobin, it does not compete with oxygen for the iron-binding positions but is bound to the protein chains of the structure.

The iron ion may be either in the Fe2+ or in the Fe3+ state, but ferrihemoglobin (methemoglobin) (Fe3+) cannot bind oxygen.(9) In binding, oxygen temporarily and reversibly oxidizes (Fe2+) to (Fe3+) while oxygen temporarily turns into the superoxide ion, thus iron must exist in the +2 oxidation state to bind oxygen. If superoxide ion associated to Fe3+ is protonated, the hemoglobin iron will remain oxidized and incapable of binding oxygen. In such cases, the enzyme methemoglobin reductase will be able to eventually reactivate methemoglobin by reducing the iron center.

In adult humans, the most common hemoglobin type is a tetramer (which contains four subunit proteins) called hemoglobin A, consisting of two α and two β subunits non-covalently bound, each made of 141 and 146 amino acid residues, respectively. This is denoted as α2β2. The subunits are structurally similar and about the same size. Each subunit has a molecular weight of about 16,000 daltons, for a total molecular weight of the tetramer of about 64,000 daltons (64,458 g/mol). Thus, 1 g/dL = 0.1551 mmol/L. Hemoglobin A is the most intensively studied of the hemoglobin molecules.

In human infants, the hemoglobin molecule is made up of 2 α chains and 2 γ chains. The gamma chains are gradually replaced by β chains as the infant grows. (10)

Oxygen saturation in general, hemoglobin can be saturated with oxygen molecules (oxyhemoglobin), or desaturated with oxygen molecules (deoxyhemoglobin). Oxyhemoglobin is formed during physiological respiration when oxygen binds to the heme component of the protein hemoglobin in red blood cells. This process occurs in the pulmonary capillaries adjacent to the alveoli of the lungs. The oxygen then travels through the blood stream to be dropped off at cells where it is utilized as a terminal electron acceptor in the production of ATP by the process of oxidative phosphorylation. It does not, however, help to counteract a decrease

in blood pH. Ventilation, or breathing, may reverse this condition by removal of carbon dioxide, thus causing a shift up in pH. Hemoglobin exists in two forms, a taut (tense) form (T) and a relaxed form (R). Various factors such as low pH, high CO2 and high 2, 3 BPG at the level of the tissues favor the taut form, which has low oxygen affinity and releases oxygen in the tissues. Conversely, a high pH, low CO2, or low 2, 3 BPG favors the relaxed form, which can better bind oxygen. The partial pressure of the system also affects O2 affinity where, at high partial pressures of oxygen (such as those present in the alveoli), the relaxed (high affinity, R) state is favoured. Inversely, at low partial pressures (such as those present in respiring tissues), the (low affinity, T) tense state is favoured. Additionally, the binding of oxygen to the iron (II) heme pulls the iron into the plane of the porphyrin ring, causing a slight conformational shift. The shift encourages oxygen to bind to the three remaining heme units within hemoglobin (thus, oxygen binding is cooperative).

Deoxygenated hemoglobin is the form of hemoglobin without the bound oxygen. The absorption spectra of oxyhemoglobin and deoxyhemoglobin differ. The oxyhemoglobin has significantly lower absorption of the 660 nm wavelength than deoxyhemoglobin, while at 940 nm its absorption is slightly higher. This difference is used for the measurement of the amount of oxygen in a patient's blood by an instrument called a pulse oximeter. This difference also accounts for the presentation of cyanosis, the blue to purplish color that tissues develop during hypoxia.

Hemoglobin variants

A part of the normal embryonic and fetal development. They may also be pathologic mutant forms of hemoglobin in a population, caused by variations in genetics. Some well-known hemoglobin variants, such as sickle-cell anemia, are responsible for diseases and are considered hemoglobinopathies. Other variants cause no detectable pathology, and are thus considered non-pathological variants.Gene expression of hemoglobin before and after birth. Also identifies the types of cells and organs in which the gene expression .Variant forms that cause disease:

*Hemoglobin D-Punjab – (α2βD2) – A variant form of hemoglobin.

*Hemoglobin H (β4) – A variant form of hemoglobin, formed by a tetramer of β chains, which may be present in variants of α thalassemia.

*Hemoglobin Barts (γ4) – A variant form of hemoglobin, formed by a tetramer of γ chains, which may be present in variants of α thalassemia.

*Hemoglobin S (α2βS2) – A variant form of hemoglobin found in people with sickle cell disease. There is a variation in the β-chain gene, causing a change in the properties of hemoglobin, which results in sickling of red

blood cells.

*Hemoglobin C (α2βC2) – Another variant due to a variation in the β-chain gene. This variant causes a mild chronic hemolytic anemia.

*Hemoglobin E (α2βE2) – Another variant due to a variation in the β-chain gene. This variant causes a mild chronic hemolytic anemia.

*Hemoglobin AS – A heterozygous form causing sickle cell trait with one adult gene and one sickle cell disease gene

*Hemoglobin SC disease – A compound heterozygous form with one sickle gene and another encoding Hemoglobin C.

*Hemoglobin Hopkins-2 - A variant form of hemoglobin that is sometimes viewed in combination with Hemoglobin S to produce sickle cell disease.

Plasma

About 55% of blood is blood plasma, a fluid that is the blood's liquid medium, which by itself is straw-yellow in color. The blood plasma volume totals of 2.7–3.0 liters (2.8–3.2 quarts) in an average human. It is essentially an aqueous solution containing 92% water, 8% blood plasma proteins, and trace amounts of other materials. Plasma circulates dissolved nutrients, such as glucose, amino acids, and fatty acids (dissolved in the blood or bound to plasma proteins), and removes waste products, such as carbon dioxide, urea, and lactic acid.

Other important components include: Serum albumin, Blood-clotting factors (to facilitate coagulation), and Immunoglobulin's (antibodies), Lipoprotein particles, various electrolytes (mainly sodium and chloride)

The term serum refers to plasma from which the clotting proteins have been removed. Most of the proteins remaining are albumin and immunoglobulins.

PH values: Blood pH is regulated to stay within the narrow range of 7.35 to 7.45, making it slightly basic. Blood that has a pH below 7.35 is too acidic, whereas blood pH above 7.45 is too basic. Blood pH, partial pressure of oxygen (pO2), partial pressure of carbon dioxide (pCO2), and bicarbonate ($HCO3^-$) are carefully regulated by a number of homeostatic mechanisms, which exert their influence principally through the respiratory system and the urinary system to control the acid-base balance and respiration. An arterial blood gas test measures these. Plasma also circulates hormones transmitting their messages to various tissues. The list of normal reference ranges for various blood electrolytes is extensive.

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Haematopoiesis

Normal Haematopoiesis

Haematopoiesis (from Greek αἷμα, «blood» and ποιεῖν "to make"; also hematopoiesis in American English; sometimes also haemopoiesis or hemopoiesis) is the formation of blood cellular components. All cellular blood components are derived from haematopoietic stem cells (1) in a healthy adult person, approximately 1011–1012 new blood cells are produced daily in order to maintain steady state levels in the peripheral circulation.

Haematopoietic stem cells (HSCs)

Haematopoietic stem cells (HSCs) reside in the medulla of the bone (bone marrow) and have the unique ability to give rise to all of the different mature blood cell types and tissues. HSCs are self-renewing cells: when they differentiate, at least some of their daughter cells remain as HSCs, so the pool of stem cells is not depleted.This phenomenon is called asymmetric division. The other daughters of HSCs (myeloid and lymphoid progenitor cells) can follow any of the other differentiation pathways that lead to the production of one or more specific types of blood cell, but cannot renew themselves. The pool of progenitors is heterogeneous and can be divided into two groups; long-term self-renewing HSC and only transiently self-renewing HSC, also called short-terms. (2) This is one of the main vital processes in the body.

All blood cells are divided into three lineages:

i) Red blood cells, also called erythrocytes, are the oxygen-carrying cells. Erythrocytes are functional and are released into the blood. The number of reticulocytes, immature red blood cells, gives an estimate of the rate of erythropoiesis.

ii) Lymphocytes are the cornerstone of the adaptive immune system. They are derived from common lymphoid progenitors. The lymphoid lineage is composed of T-cells, B-cells and natural killer cells.

iii) Cells of the myeloid lineage, which include granulocytes, megakaryocytes and macrophages, are derived from common myeloid progenitors, and are involved in such diverse roles as innate immunity and blood clotting.

Granulopoiesis (or granulocytopoiesis) is haematopoiesis of granulocytes, except of mast cells which are granulocytes but with an extramedullar maturation. Megakaryocytopoiesis is haematopoiesis of megakaryocytes.

Sites of haematopoiesis

In developing embryos, blood formation occurs in aggregates of blood cells in the yolk sac, called blood islands. As development progresses, blood formation occurs in the spleen, liver and lymph nodes. When bone marrow develops, it eventually assumes the task of forming most of the blood cells for the entire organism. However, maturation, activation, and some proliferation

of lymphoid cells occurs in the spleen, thymus, and lymph nodes. In children, haematopoiesis occurs in the marrow of the long bones such as the femur and tibia. In adults, it occurs mainly in the pelvis, cranium, vertebrae, and sternum.

Extramedullary In some cases, the liver, thymus, and spleen may resume their haematopoietic function, if necessary. This is called extramedullary haematopoiesis. It may cause these organs to increase in size substantially. During fetal development, since bones and thus the bone marrow develop later, the liver functions as the main haematopoetic organ. Therefore, the liver is enlarged during development.

In some vertebrates, haematopoiesis can occur wherever there is a loose stroma of connective tissue and slow blood supply, such as the gut, spleen or kidney.

Erythropoiesis

Erythropoiesis (from Greek 'erythro' meaning "red" and 'poiesis' meaning "to make") is the process which produces red blood cells (erythrocytes). It is stimulated by decreased O2 in circulation, which is detected by the kidneys, which then secrete the hormone erythropoietin (3). This hormone stimulates proliferation and differentiation of red cell precursors, which activates increased erythropoiesis in the hemopoietic tissues, ultimately producing red blood cells (erythrocytes).In postnatal birds and mammals (including humans), this usually occurs within the red bone marrow. In the early fetus, erythropoiesis takes place in the mesodermal cells of the yolk sac. By the third or fourth month, erythropoiesis moves to the liver (4). After seven months, erythropoiesis occurs in the bone marrow. Increased level of physical activity can cause an increase in erythropoiesis. However, in humans with certain diseases and in some animals, erythropoiesis also occurs outside the bone marrow, within the spleen or liver. This is termed extramedullary erythropoiesis. The bone marrow of essentially all the bones produces red blood cells until a person is around five years old. The tibia and femur cease to be important sites of hematopoiesis by about age 25; the vertebrae, sternum, pelvis and ribs, and cranial bones continue to produce red blood cells throughout life.

Erythrocyte differentiation

In the process of red blood corpuscle maturation, a cell undergoes a series of differentiations. The following stages of development all occur within the bone marrow: A hemocytoblast, a multipotent hematopoietic stem cell, becomes a common myeloid progenitor or a multipotent stem cell, and then a unipotent stem cell, then a pronormoblast, also commonly called aproerythroblast or a rubriblast. This becomes a basophilic or early normoblast, also commonly called an erythroblast, then apolychromato-

philic or intermediate normoblast, then an orthochromatic or late normoblast. At this stage the nucleus is expelled before the cell becomes a reticulocyte. And so in newly circulating red blood cells there are about 1% reticulocytes. After one to two days, these ultimately become "erythrocytes" or mature red blood cells. These stages correspond to specific appearances of the cell when stained with Wright's stain and examined by light microscopy, and correspond to other biochemical changes.

In the process of maturation, a basophilic pronormoblast is converted from a cell with a large nucleus and a volume of 900 fL to an enucleated disc with a volume of 95 fL. By the reticulocyte stage, the cell has extruded its nucleus, but is still capable of producing hemoglobin.

Vitamin B12 (cobalamin) and Vitamin B9 (Folic acid) are Essential for the maturation of red blood cells. Lack of either causes maturation failure in the process of erythropoiesis, which manifests clinically as reticulocytopenia (an abnormally low amount of reticulocytes).

Characteristics seen in erythrocytes during erythropoiesis: As they mature, a number of erythrocyte characteristics change: The overall size of the erythroid precursor cell reduces with the cytoplasmic to nucleus (C: N) ratio increasing. The nuclear diameter decreases and chromatin condenses with the staining reaction progressing from purplish red to dark blue at the final nuclear stage of orthochromatic erythroblast, prior to nuclear ejection. The colour of the cytoplasm changes from blue at proerthroblast and basophillic stages to a pinkish red as a result of the increasing expression of haemoglobin as the cell develops. Initially, the nucleus is large in size and contains open chromatin. But, as red blood cells mature, the size of the nucleus decreases, until it finally disappears with the condensation of the chromatin material.

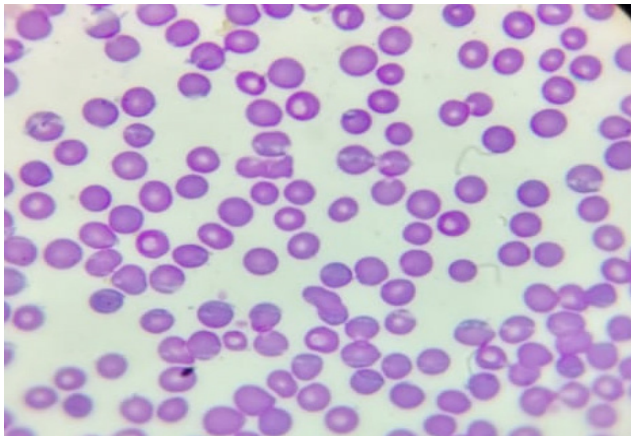
Regulation of erythropoiesis

A feedback loop involving erythropoietin helps regulate the process of erythropoiesis so that, in non-disease states, the production of red blood cells is equal to the destruction of red blood cells and the red blood cell number is sufficient to sustain adequate tissue oxygen levels. Erythropoietin is produced in the kidney and liver in response to low oxygen levels. In addition, erythropoietin is bound by circulating red blood cells; low circulating numbers lead to a relatively high level of unbound erythropoietin, which stimulates production in the bone marrow.

Recent studies have also shown that the peptide hormone hepcidin may play a role in the regulation of hemoglobin production, and thus affect erythropoiesis. The liver produces hepcidin. Hepcidin controls iron absorption in the gastrointestinal tract and iron release from reticuloendothelial tissue. Iron must be released from macrophages in

the bone marrow to be incorporated into the heme group of hemoglobin in erythrocytes. There are colony forming units that the cells follow during their formation. These cells are referred to as the committed cells including the granulocyte monocyte colony forming units.

The secretion of hepcidin is inhibited by another hormone, erythroferrone, produced by erythroblasts in response to erythropoietin, and identified in 2014 (5). It appears that this links erythropoietin-driven eyrthropoiesis with the iron mobilization needed for hemoglobin synthesis. Loss of function of the erythropoietin receptor or JAK2 in mice cells causes failure in erythropoiesis, so production of red blood cells in embryos and growth is disrupted. If there is no systemic feedback inhibition, for example, the diminishment or absence of suppressors of cytokine signaling proteins, giantism may result.



(Figure 2-1) Blood film stained by Romanowsky stain shows human Red blood cells.

Leukopoiesis

Leukopoiesis is a form of hematopoiesis in which white blood cells (WBC, or leukocytes) are formed in bone marrow located in bones in adults and hematopoietic organs in the fetus. White blood cells, indeed all blood cells, are formed from the differentiation of pluripotent hematopoietic stem cells which give rise to several cell lines with unlimited differentiation potential. These immediate cell lines, or colonies, are progenitors of red blood cells (erythrocytes), platelets (megakaryocytes), and the two main groups of WBCs, myelocytes and lymphocytes.

Myelopoiesis

In hematology, myelopoiesis in the broadest sense of the term is the production of bone marrow and of all cells that arise from it, namely, all blood cells. But in a narrower sense that is also commonly used, myelopoiesis is the regulated formation specifically of myeloid leukocytes (myelocytes), including eosinophilic granulocytes, basophilic granulocytes, neutrophilic granulocytes, and monocytes.

The common myeloid progenitor can differentiate in the bone marrow into red blood cells and megakaryocytes (leading to platelets) as well as mast cells and myeloblasts, the latter leading to the myelocytic line (granulocytes) and to monocytes, macrophages, and dendritic cells of the innate immune system. The granulocytes, also called polymorphonuclear leukocytes because of their

multilobed nuclei, are three short lived cell types including eosinophils, basophils, and neutrophils. A granulocyte differentiates into a distinct cell type by a process called granulopoiesis. In this process it first transforms from a common myeloblast (myeloid progenitor) to a common promyelocyte. This promyelocyte gives rise to a unique myelocyte that for the first time can be classified as an eosinophil, basophil, or neutrophil progenitor based on the histological staining affinity (eosinophilic, basophilic, or neutral granules). The unique myelocyte next differentiates into a metamyelocyte and then a band cell, with a "C" shaped nucleus, before becoming a mature eosinophil, basophil, or neutrophil. Macrophages come from monoblast progenitors that differentriate into promonocytes, which mature into monocytes. Monocytes eventually enter the tissues and become macrophages.

Lymphopoiesis

Lymphopoiesis (lĭm'fō-poi-ē›sĭs) (or lymphocytopoiesis) is the generation of lymphocytes, one of the five types of white blood cell (WBC) (6). It is more formally known as lymphoid hematopoiesis.

"Pathosis in lymphopoiesis leads to any of various lymphoproliferative disorders, such as the lymphomas and lymphoid leukemias."

Lymphopoiesis Acronyms

• B-NK Progenitor for B and NK

• CB Cord blood

• CFU Colony-forming Unit

• CLP Common Lymphoid Progenitor

• CMP Common Myeloid Progenitor

• DC Dendritic Cell (Myeloid or Lymphoid)

• ELP Early Lymphoid Progenitors

• ETP the most primitive cells in the thymus are the Early Thymocyte Progenitors

• G-CSF Granulocyte Colony Stimulating Factor

• GM-CSF Granulocyte Macrophage Colony Stimulating Factor

• GMP Granulocyte Macrophage Progenitor;

• HSC pluripotential Hemopoietic Stem Cell

• MDC combined Macrophage and DC progenitor potential

• MEP megakaryocytic and erythroid progenitor

• MLP Multi-lymphoid Progenitor potential, any progenitor minimally able to give rise to B cells, T cells and NK cells

• MPP Multipotent Progenitor

• Notch Notch signaling pathway re T cell commitment from progenitors

Lymphopoiesis can be viewed in a mathematical sense as a recursive process of cell division and also as a process of differentiation, measured by changes to the properties

of cells.

Given that lymphocytes arise from specific types of limited stem cells - which we can call P (for Progenitor) cells - such cells can divide in several ways. These are general principles of limited stem cells.

Considering the P as the 'mother' cell, but not a true stem cell, it may divide into two new cells, which are themselves identical, but differ to some degree from the mother. Or the mother cell P may divide unequally into two new daughter cells both of which differ from each other and also from the mother.

Any daughter cell will usually have new specialized abilities and if it is able to divide it will form a new sub-lineage. The difference of a daughter cell from the mother may be great, but it could also be much less, even subtle. What the P mother cell does not do is divide into two new P mother cells or a mother and a daughter; this is a matter of observation as such limited progenitor cells are known to not self-renew.

There is a sort of exception when daughter cells at some level of the lineage may divide several times to form more seemingly identical cells, but then further differentiation and division will inevitably occur, until a final stage is reached in which no further division can occur and the cell type lineage is finally mature. An example of maturity is a plasma cell, from the B cell lineage, which produces copious antibody, but cannot divide and eventually dies after a few days or weeks.

The progenitor CLP of the mouse or the progenitor MLP of the human differentiates into lymphocytes by first becoming a lymphoblast. It then divides several more times to become a prolymphocyte that has specific cell-surface markers unique to either a (1) T cell or (2) B cell. The progenitor can also differentiate into (3) natural killer cells (NK) and (4) dendritic cells.

T Cells, B Cells and NK Cells (and all other innate lymphoid cells) are unique to the lymphocyte family, but dendritic cells are not. Dendritic cells of identical appearance but different markers are spread throughout the body, and come from either lymphoid or myeloid lineages, but these cells may have somewhat different tasks and may take up lodging preferentially in different locations. This is now an open question; also, the different dendritic cell lineages may have different 'tasks' and stay in different 'locations.

T and B lymphocytes are indistinguishable histologically that is, under a light microscope they cannot be told apart. Indeed, the inactive B and T cells are so featureless with few cytoplasmic organelles and mostly inactive chromatin that until the 1960s textbooks could describe these cells, now the central focus of immunology, as having no known function!!

However T and B lymphocytes are very distinct cell lineages and they 'grow up' in dif-

ferent places in the body. They perform quite different (although co-operative) functions in the body. No evidence has ever been found that T and B cells can ever interconvert. T and B cells are biochemically distinct and this is reflected in the differing markers and receptors they possess on their cell surfaces. This seems to be true in all vertebrates, although there are many differences in the details between the species.

Regardless of whether the CLP (mouse) or MLP or a small closely related set of progenitor cells take credit for generating the profusion of lymphocytes, the same lymphoid progenitors can still generate some cells that are clearly identifiably myeloid.

Lymphopoiesis for T cells

T cells are formed in bone marrow then migrate to the cortex of the thymus to undergo maturation in an antigen-free environment for about one week where a mere 2-4% of the T cells succeed. The remaining 96-98% of T cells die by apoptosis and are phagocytosed by macrophages in the thymus. So many thymocytes (T cells) die during the maturation process because there is intensive screening to make sure each thymocyte has the ability to recognize self-peptide:self-MHC complex and for self-tolerance (7). The apoptosed thymocyte dies and is quickly recycled.

Upon maturity, there are several forms of thymocytes including:

*T-helper (needed for activation of other cells such as B cells and macrophages).

*T-cytotoxic (which kill virally infected cells).

*T-memory (T cells that remember antigens previously encountered).

*T-suppressor cells (which moderate the immune response of other leukocytes). Also called T-regulatory cells (Tregs)

When T-Cells become activated they undergo a further series of developments. A small, resting T lymphocyte rapidly undergoes blastogenic transformation into a large lymphocyte (13–15µm). This large lymphocyte (known in this context as a lymphoblast) then divides several times to produce an expanded population of medium (9–12µm) and small lymphocytes (5–8µm) with the same antigenic specificity. Final activated and differentiated T lymphocytes are once again morphologically indistinguishable from a small, resting lymphocyte. Thus the following developmental states may be noticed in sequence in blood tests: Prolymphocyte>Large lymphocyte>Small lymphocyte.

T cell development

Unlike other lymphoid lineages, T cell development occurs almost exclusively in the thymus. T-lymphopoiesis does not occur automatically but requires signals generated from the thymic stromal cells. Several stages at which specific regulators and growth factors are required for T cell development to

proceed have been defined. Later in T cell development and its maturation these same regulatory factors again are used to influence T cell specialization.

T cells are unique among the lymphocyte populations in their ability to further specialize as mature cells and become yet more mature. And T Cells come in many flavors, for example: the conventional TcRαβ T cells; the so-called unconventional TcRγδ T cells; NKT cells; and T regulatory cells (Treg). Details regarding the developmental and life cycle of the unconventional T cells are less well-described compared to the conventional T cells.

Stages of T cell maturation

Stage One: Thymic Migration: Multipotent lymphoid progenitors (MLP) enter the T cell pathway as they immigrate to the thymus. The most primitive cells in the thymus are the early thymocyte progenitors (ETP), which retain all lymphoid and myeloid potential but exist only transiently, rapidly differentiating into T and NK lineages.

Stage Two: Proliferative Expansion and T Lineage Commitment: Final commitment to the T cell lineage occurs within the thymic microenvironment, the microscopic structures of the thymus where T cells are nurtured. The most primitive T cells retain pluripotential ability and can differentiate into cells of the myeloid or lymphoid lineages (B cells, DC, T cells, or NK cells). More differentiated double negative T cells (DN2 cells) have more limited potentiality but are not yet fully restricted to the T cell lineage (they can still develop into DC, T cells, or NK cells). Later on, they are fully committed to the T cell lineage- when thymoctyes expressing Notch1 receptors engage thymic stromal cells expressing Notch1 ligands, the thymocytes become finally committed to the T-cell lineage.

With the commitment to the T cell lineage, begins a very complex process known as TcR gene rearrangement. This creates an enormous diversity of T cells bearing antigen receptors. Afterward some T cells leave the thymus to migrate to the skin and mucosae.

Stage Three: β-Selection, This process selects for cells that have successfully rearranged their TCR-β chain locus. The β chain then pairs with the surrogate chain, pre-Tα, and produces a pre-TCR, which forms a complex with CD3 molecules. This complex leads to the survival, proliferation, arrest in further β chain loci rearrangement, and further differentiation by up-regulation and expression of CD4 and CD8, these cells are termed double positive (DP) cells. Cells that do not undergo beta-selection die by apoptosis.

Stage Four: T cell Receptors Selection: Only 2% to 3% of the differentiating thymocytes, those that express TcR capable of interaction with MHC molecules, but tolerant to self-peptides, survive the Stage Four selection process.

Stage Five: Continuing Differentiation in the Periphery: It was previously believed that the human thymus remained active as the site of T cell differentiation only until early adulthood and that later in adult life the thymus atrophies, perhaps even vanishing. Recent reports indicate that the human thymus is active throughout adult life. Thus several factors may contribute to the supply of T cells in adult life: generation in the thymus, extra-thymic differentiation, and the fact that memory T cells are long-lived and survive for decades.

T cell types

Unconventional T cells the thymus also gives rise to the so-called 'unconventional T cells' such at γδ T cells, Natural Killer T cells (NKT) and regulatory T cells (Treg).

γδT cells represent only 1% to 5% of the circulating T cells, but are abundant in the mucosal immune system and the skin, where they represent the dominant T cell population. These 'non-MHC restricted T cells' are involved in specific primary immune responses, tumor surveillance, immune regulation and wound healing(8).

Several differences between αβ and γδ T cell development have been described. They emigrate from the thymus in "waves" of clonal populations, which home to discrete tissues. For example, one kind is found in the peripheral blood while another predominates in the intestinal tract.

Natural Killer T cells Human NKT cells are a unique population and are thought to play an important role in tumor immunity and immunoregulation.

T Regulatory cells "Tregs" are considered as naturally occurring regulatory T cells. Tregs comprised about 5% of the circulating CD4+ T cells. These cells are thought to possess an important autoimmunity property by regulating 'autoreactive' T cells in the periphery.

Lymphopoiesis for B cells

B cells are formed and mature in bone marrow (and spleen).These B cells then leave the bone marrow and migrate to peripheral lymphoid tissues, such as a lymph node. Once in a secondary lymphoid organ the B cell can be introduced to an antigen that it is able to recognize.

Through this antigen recognition and other cell interactions the B cell becomes activated and then divides and differentiates to become a plasma cell. The plasma cell, a B cell end product, is a very active antibody-secreting cell that helps protect the body by attacking and binding to antigen.

Even after many decades of research, some controversy remains as to where B cells mature and 'complete their education', with the possibility remaining that the site may also partially be peri-intestinal lymphoid tissues(9).

B lymphopoiesis occurs exclusively in the bone marrow and B lymphocytes are made

continuously throughout life there in a 'microenvironment' composed of stromal cells, extracellular matrix, cytokines and growth factors, which are critical for proliferation, differentiation, and survival of early lymphocyte and B-lineage precursors.

The relative proportion of precursor B cells in the bone marrow remains rather constant throughout the life span of an organism. There are stages such as Pre-B-I cells (5% to 10% of the total); Pre-B-II cells (60% to 70%) while the remaining 20% to 25% are immature B cells. Most textbooks say that B Cells mature in the bone marrow but, generally, immature B cells migrate to the spleen for 'higher education' of some sort where they go through transitional stages before final maturation.

B lymphocytes are identified by the presence of soluble immunoglobulin G (IgG). This is the most common protective immunoglobulin in the adult body. After antigenic stimulation, B cells differentiate into plasma cells that secrete large quantities of soluble IgG. This is the final stage of B lymphopoiesis but it is the clincher because the plasma cells must either issue antibody close to a source of infection, or disseminate it in the blood to fight an infection at a distance or in an inaccessible part of the body.

A generally regarded valid map of B cell lymphopoiesis is as follows in sequence, in two parts with the first being in the bone marrow and the second in the spleen. The development process in the bone marrow occurs in Germinal Centers.

In the bone marrow: Pro-B>Pre-B-I>Pre-B-II large>Pre-B-II small.

In the spleen: T1>T2/T3> (Marginal Zone (MZ); B-1; B-2)>B-2 further differentiate into :(Germinal Center (GC); Memory; Plasma)

Lymphopoiesis for NK cells

NK cells, which lack antigen specific receptors, develop in the bone marrow. After maturation and release from the marrow they circulate in the blood through their lifetime seeking opportunity. The opportunity they seek is to encounter and recognize and then kill abnormal cells such as cancer or virally infected cells. It is well known that lymphocytes never have granules or at least not granules that are readily visible even upon staining. However NK cells are the exception. They do have numerous granules which provide their ability to kill cells and these granules are why NK cells have an alternate name, LGL, Large Granular Lymphocytes.

NK cells not only have a catchy movie-title name (Natural Killer) but are also the only lymphocytes considered part of the innate immune system (in contrast to the adaptive immune system. Yet they are much more closely related to T cells (part of the adaptive immune system) than to other cells of the innate immune system. NK cells not only share many surface markers, functions and activities in common with T Cells, they also

arise from a common T/NK progenitor. The T/NK precursor is also believed to be the source of a subpopulation of lymphoid DC.

NK cells have a definition 'barcode' as CD3, CD16+, CD56t lymphocytes. (See Barcode Section of this article). NK progenitors can be found mainly in the thymus (mouse), but the thymus is not absolutely required for NK development. Probably NK cells can develop in a variety of organs but the major site of NK cell development is not known.

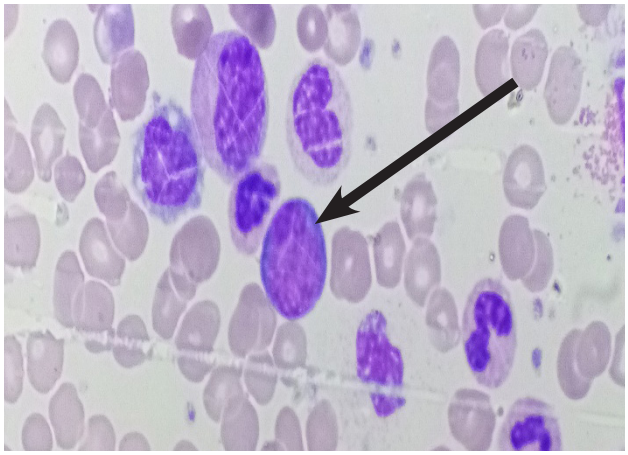
In humans, the majority (85–90%) of the NK cells have a high cytolytic capacity (the ability to lyse cells). A smaller subset (10–15%) called NK 'CD56 bright' is chiefly responsible for cytokine production and has enhanced survival. Traveling to lymph nodes the 'CD56 bright' NK cells differentiate again into mature NK cells which express killer cell immunoglobulin-like receptors (KIR), natural cytotoxicity receptors (NCR), and critical adhesion molecules.

Lymphopoiesis for dendritic cells

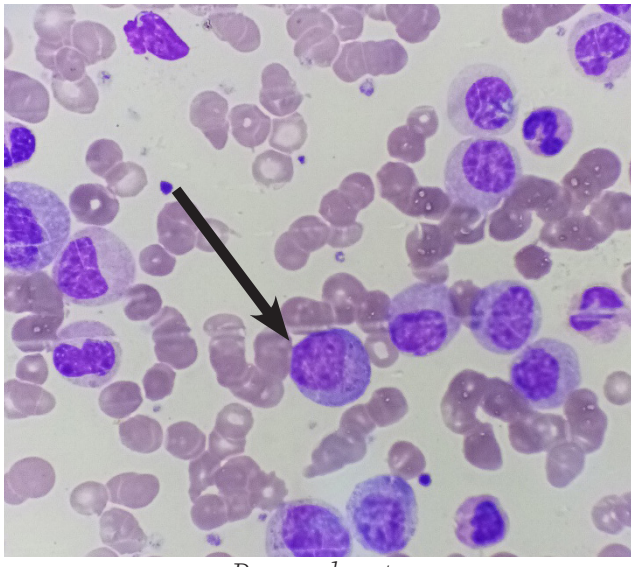
Dendritic cell is usually abbreviated DC or DCs. The process by which CLP cells may differentiate to generate dendritic cells of lymphoid lineage is not yet well defined.

DCs are highly specialized and efficient antigen-presenting cells. Cells identical in appearance come both from a myeloid lineage (referred to as myeloid dendritic cells) and also from a lymphoid lineage (referred to as plasmacytoid dendritic cells).

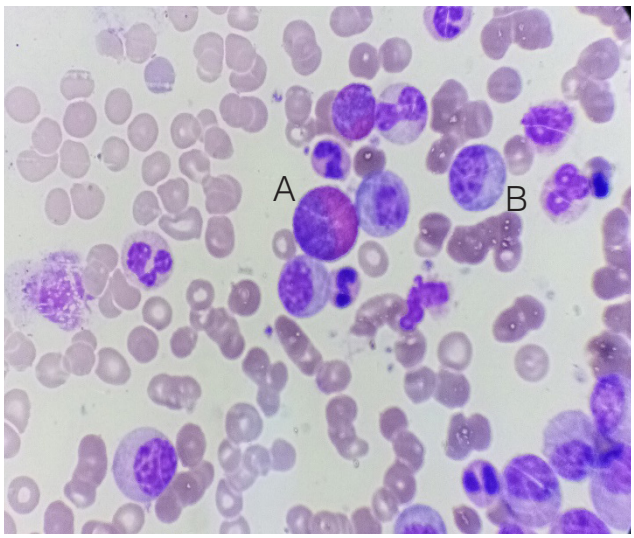
The development and regulation of DC is not well-characterized. While the DC precursors have been identified in the human fetal liver, thymus, and bone marrow, during adult life DC are thought to be produced only from the bone marrow and released into the blood to wander and settle down. Overall a large number of DC of varying types are dispatched throughout the body, especially at epithelia such as skin, to monitor invaders and nibble their antigens.



Myeloblaste

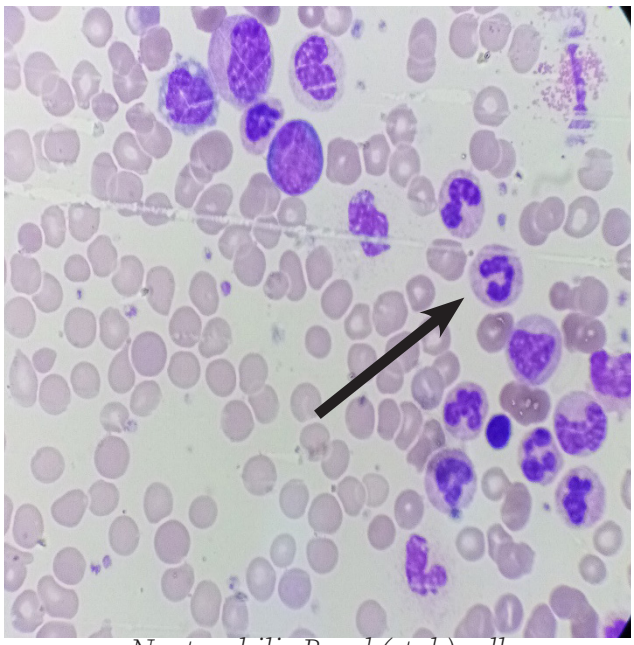


Pormyelocyte

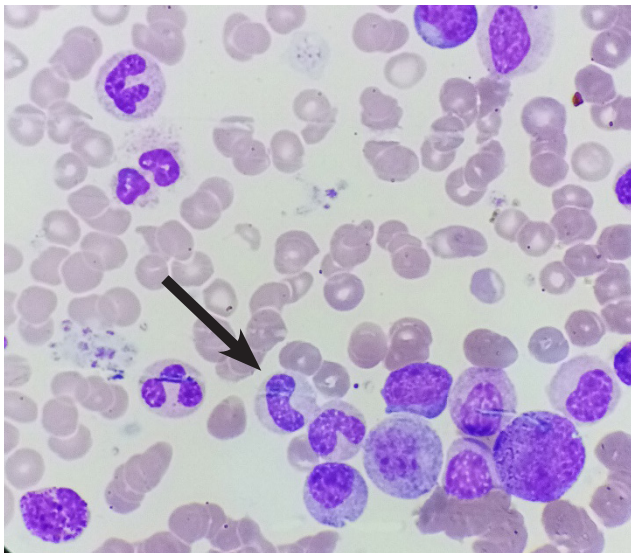


A: Eosinophilic myelocyte

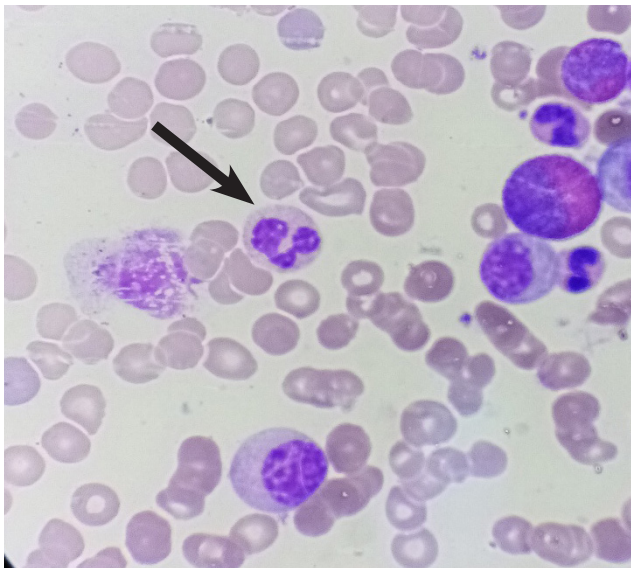
B: neutrophilic myelocyte



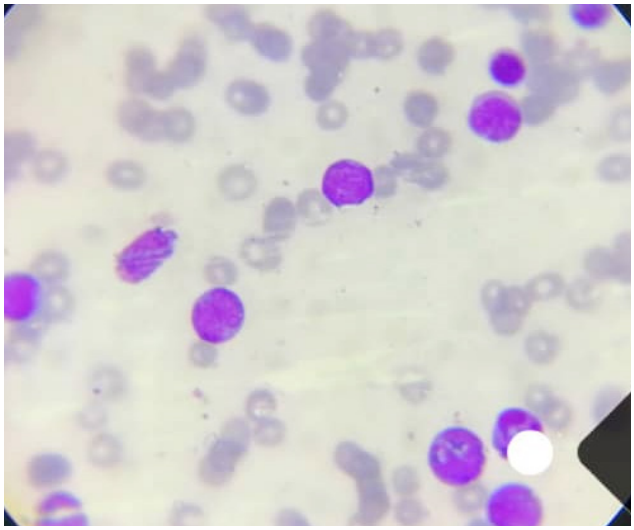
Neutrophilic Band (stab) cell



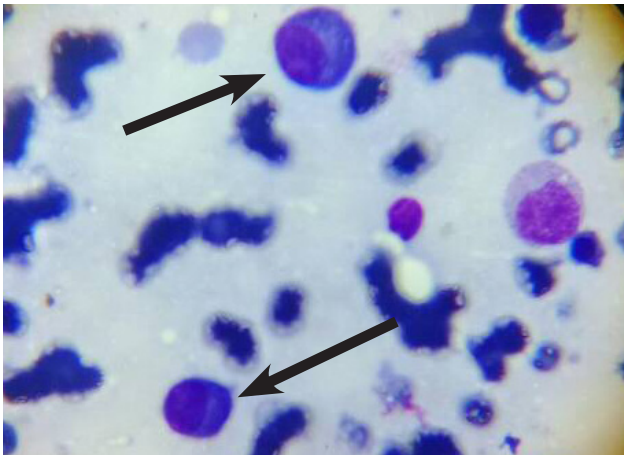
Metamyelocyte



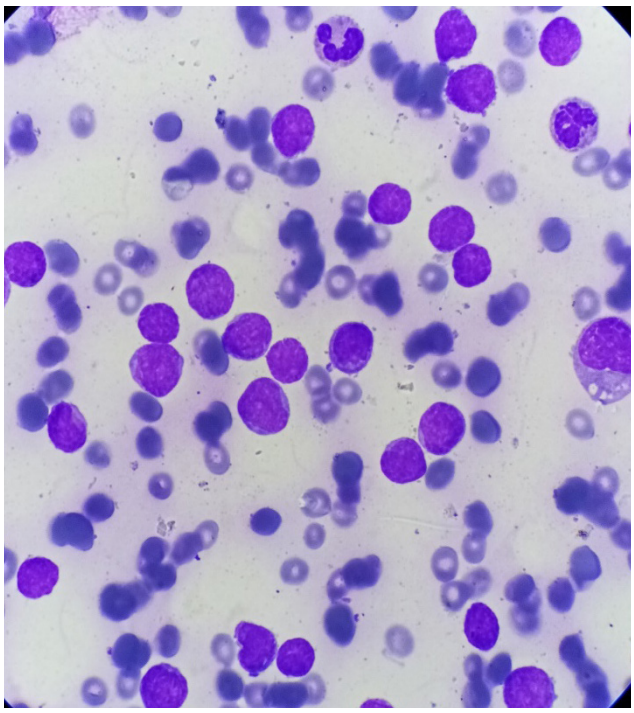
Mature neutrophil



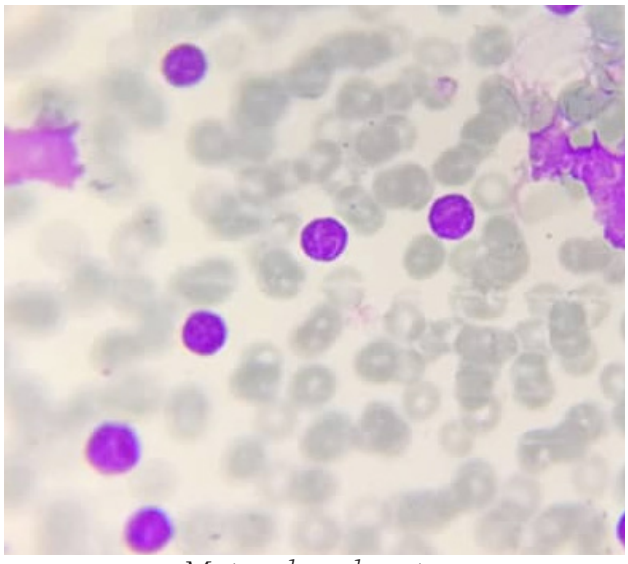
Lymphoblasts



Pasma cells



Prolymphocytes



Mature lymphocytes

(Figure 2-2) shows: myeloblast, promyelocyte,Eosinopilic myelocyte ,Metamyelocyte ,neutrophilic myelocyte, Neutropilic Band (stab) cell, Mature neutrophil, lymphoblast, prolymphocyte, mature lymphocyte and plasma cell respectively.

Thrombopoiesis

Thrombopoiesis is the process of thrombocyte generation. Thromobocytes are ligations of the cytoplasm from megakaryocytes. A single megakaryocyte can give rise to thousands of thrombocytes.

The term "thrombocytopoiesis" is sometimes used to emphasize the cellular nature.

Thrombopoietin stimulates megakaryopoiesis, the process of megakaryocyte maturation and differentiation. Thrombopoietin, upon release, binds to its receptor, c-mpl, found on megakaryocyte progenitor cells. Following binding, intracellular signalling leads to megakaryocyte growth, maturation, membrane stability, platelet granule formation and the demarcation of the cytoplasm into regions destined to fragment into mature platelets. These "proplatelet processes" further fragment into platelets. This last step of proplatelet process and platelet formation, in vitro, has been shown to be independent of thrombopoietin.

Blood coagulation and normal hemostasis

Coagulation, also known as clotting, is the process by which blood changes from a liquid to a gel, forming a blood clot. It potentially results in hemostasis, the cessation of blood loss from a damaged vessel, followed by repair. The mechanism of coagulation involves activation, adhesion and aggregation of platelets, as well as deposition and maturation of fibrin.

Coagulation begins almost instantly after an injury to the blood vessel has damaged the endothelium lining the blood vessel. Exposure of blood to the subendothelial space initiates two processes: changes in platelets, and the exposure of subendothelial tissue factor to plasma Factor VII, which ultimately leads to cross-linked fibrin formation. Platelets immediately form a plug at the site of injury; this is called primary hemostasis. Secondary hemostasis occurs simultaneously: additional coagulation (clotting) factors beyond Factor VII respond in a cascade to form fibrin strands, which strengthen the platelet plug (10).

Disorders of coagulation are disease states which can result in bleeding -hemorrhage or bruising - or obstructive clotting - thrombosis.

Coagulation is highly conserved throughout biology. In all mammals, coagulation involves both a cellular (platelet) and a protein (coagulation factor) component. The system in humans has been the most extensively researched and is the best understood.

Platelet activation

When the endothelium is damaged, the normally isolated, underlying collagen is exposed to circulating platelets, which bind directly to collagen with collagen-specific glycoprotein Ia/IIa surface receptors. This adhesion is strengthened further by von Willebrand factor (vWF), which is released from the endothelium and from platelets; vWF forms additional links between the platelets' glycoprotein Ib/IX/V and the collagen fibrils. This localization of platelets to the extracellular matrix promotes collagen interaction with platelet glycoprotein VI. Binding of collagen to glycoprotein VI triggers a signaling cascade that results in activation of platelet integrins. Activated integrins mediate tight binding of platelets to the extracellular matrix. This process adheres platelets to the site of injury.

Activated platelets release the contents of stored granules into the blood plasma. The granules include ADP, serotonin, platelet-activating factor (PAF), vWF, platelet factor 4, and thromboxane A2 (TXA2), which, in turn, activate additional platelets. The granules' contents activate a Gq-linked protein receptor cascade, resulting in increased calcium concentration in the platelets' cytosol. The calcium activates protein kinase C, which, in turn, activates phospholipase A2 (PLA2). PLA2 then modifies the integrin membrane glycoprotein IIb/IIIa, increasing its affinity to bind fibrinogen. The activated platelets change shape from spherical to stellate, and the fibrinogen cross-links with glycoprotein IIb/IIIa aid in aggregation of adjacent platelets (completing primary hemostasis). Coagulation cascade

The coagulation cascade of secondary hemostasis has two initial pathways which lead to fibrin formation. These are the contact activation pathway (also known as the intrinsic pathway), and the tissue factor path-

way (also known as the extrinsic pathway), which both lead to the same fundamental reactions that produce fibrin. It was previously thought that the two pathways of coagulation cascade were of equal importance, but it is now known that the primary pathway for the initiation of blood coagulation is the tissue factor (extrinsic) pathway. The pathways are a series of reactions, in which a zymogen (inactive enzyme precursor) of a serine protease and its glycoprotein co-factor are activated to become active components that then catalyze the next reaction in the cascade, ultimately resulting in cross-linked fibrin. Coagulation factors are generally indicated by Roman numerals, with a lowercase "a" appended to indicate an active form.

The coagulation factors are generally serine proteases (enzymes), which act by cleaving downstream proteins. The exceptions are tissue factor, FV, FVIII, FXIII. Tissue factor, FV and FVIII are glycoproteins, and Factor XIII is a transglutaminase. The coagulation factors circulate as inactive zymogens. The coagulation cascade is therefore classically divided into three pathways. The tissue factor and contact activation pathways both activate the "final common pathway" of factor X, thrombin and fibrin.

The Intrinsic pathway

Is less important for initiating coagulation than the extrinsic pathway, The intrinsic pathway is very important for the amplification of the cascade.

This pathway consist of a number of proteins called factors, activating on another.

The first factor in this pathway is factor twelve "F XII" , the intrinsic pathway is known as contact pathway because "F XII" is converted to its active form "F XIIa" when it comes in contact with a negatively charged surface ,Thus "F XIIa" causes the conversion of "F XI" to "F XIa" , "FXIa" converts "F IX" to "F IXa" which with the help of "F VIIIa" (activated from "F VIII") activates "F X" to "F Xa" , "F Xa" can then convert Prothrombin "F II" to Thrombin "F IIa" with the help of "F Va" , Thrombin then converts Fibrinogen "F I" to Fibrin "F Ia" , Fibrin forms a mesh at the injury site to help reduce the blood loss.

The Extrinsic pathway

Is the most important for initiating the coagulation cascade. It begins when a protein called Tissue Factor "F III" is released from a damaged tissue, "F VII" is activated "F VIIa", then "F III" with "F VIIa" combines to activate "F X" to "F Xa", then the pathway continues on like the intrinsic pathway until Fibrin is produced.

The positive feedback effect of Thrombin in the cascade

Thrombin has a role in accelerating the production of " F X , F VIII , F VII , F V and F XIII".

The role of "F XIII" is to form a cross link

between the Fibrin strands to form a tide mesh.

Cofactors

Various substances are required for the proper functioning of the coagulation cascade: Calcium and phospholipid

Calcium and phospholipid (a platelet membrane constituent) are required for the tenase and prothrombinase complexes to function. Calcium mediates the binding of the complexes via the terminal gamma-carboxy residues on FXa and FIXa to the phospholipid surfaces expressed by platelets, as well as procoagulant microparticles or microvesicles shed from them. Calcium is also required at other points in the coagulation cascade.

Vitamin K

Vitamin K is an essential factor to a hepatic gamma-glutamyl carboxylase that adds a carboxyl group to glutamic acid residues on factors II, VII, IX and X, as well as Protein S, Protein C and Protein Z. In adding the gamma-carboxyl group to glutamate residues on the immature clotting factors, Vitamin K is itself oxidized. Another enzyme, Vitamin K epoxide reductase (VKORC), reduces vitamin K back to its active form. Vitamin K epoxide reductase is pharmacologically important as a target of anticoagulant drugs warfarin and related coumarins such as acenocoumarol, phenprocoumon, and dicumarol. These drugs create a deficiency of reduced vitamin K by blocking VKORC, thereby inhibiting maturation of clotting factors. Vitamin K deficiency from other causes (e.g., in malabsorption) or impaired vitamin K metabolism in disease (e.g., in liver failure) lead to the formation of PIVKAs (proteins formed in vitamin K absence), which are partially or totally non-gamma carboxylated, affecting the coagulation factors' ability to bind to phospholipid. Regulators

Five mechanisms keep platelet activation and the coagulation cascade in check. Abnormalities can lead to an increased tendency toward thrombosis.

Protein C

Protein C is a major physiological anticoagulant. It is a vitamin K-dependent serine protease enzyme that is activated by thrombin into activated protein C (APC). Protein C is activated in a sequence that starts with Protein C and thrombin binding to a cell surface protein thrombomodulin. Thrombomodulin binds these proteins in such a way that it activates Protein C. The activated form, along with protein S and a phospholipid as cofactors, degrades FVa and FVIIIa. Quantitative or qualitative deficiency of either (protein C or protein S) may lead to thrombophilia (a tendency to develop thrombosis). Impaired action of Protein C (activated Protein C resistance), for example by having the "Leiden" variant of Factor V or high levels of FVIII, also may lead to a thrombotic tendency. Antithrombin Edit

Antithrombin is a serine protease inhibitor (serpin) that degrades the serine proteases: thrombin, FIXa, FXa, FXIa, and FXIIa. It is constantly active, but its adhesion to these factors is increased by the presence of heparan sulfate (a glycosaminoglycan) or the administration of heparins (different heparinoids increase affinity to FXa, thrombin, or both). Quantitative or qualitative deficiency of antithrombin (inborn or acquired, e.g., in proteinuria) leads to thrombophilia.

Tissue factor pathway inhibitor (TFPI)

Tissue factor pathway inhibitor (TFPI) limits the action of tissue factor (TF). It also inhibits excessive TF-mediated activation of FVII and FX.

Plasmin

Plasmin is generated by proteolytic cleavage of plasminogen, a plasma protein synthesized in the liver. This cleavage is catalyzed by tissue plasminogen activator (t-PA), which is synthesized and secreted by endothelium. Plasmin proteolytically cleaves fibrin into fibrin degradation products that inhibit excessive fibrin formation.

Prostacyclin

Prostacyclin (PGI2) is released by endothelium and activates platelet Gs protein-linked receptors. This, in turn, activates adenylyl cyclase, which synthesizes cAMP. cAMP inhibits platelet activation by decreasing cytosolic levels of calcium and, by doing so, inhibits the release of granules that would lead to activation of additional platelets and the coagulation cascade.

Fibrinolysis

Eventually, blood clots are reorganised and resorbed by a process termed fibrinolysis. The main enzyme responsible for this process (plasmin) is regulated by various activators and inhibitors.

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Red Blood Disorders

Blood disorders

Blood disorders are conditions that impact the blood's ability to function correctly. There is a range of different types and symptoms depend on the type. However, some common symptoms include unexplained fatigue and weight loss.

Most blood disorders decrease the number of cells, proteins, platelets, or nutrients in the blood, or interfere with their function. A majority of blood disorders are caused by mutations in parts of specific genes and can be passed down in families.

A blood disorder is any condition that impacts one or more parts of the blood, usually interfering with its ability to work correctly. A blood disorder can affect one or more components of the blood. Many blood disorders take their name from the component of the blood they impact.

The following categories describe blood disorders that cause a decrease in blood components or affect their function:

Anemia – if the disorder involves red blood cells.

Leukopenia – if the disorder affects white blood cells.

Thrombocytopenia – if the disorder concerns platelets.

Categories of blood disorders that increase blood components are:

Erythrocytosis – if the disorder involves red blood cells.

Leukocytosis – if the disorder affects white blood cells.

Thrombocythemia or thrombocytosis – if the disorder concerns platelets.

Red blood cells disorders

Red blood cells (RBCs) have a special shape, size and count in a healthy human, with a little range of variation according to age, sex and other secondary factors. Any change in the normal ranges will affect the human health and cause specific signs and symptoms may be fatal if left without treatment.

When red blood cells count was below the lower limit of the normal range, this situation is called anemia. Also when it's more than the upper limit of the normal range the situation is called a polycythemia.

Anemia

Anemia is a decrease in the total amount of red blood cells (RBCs) or hemoglobin in the blood, or a lowered ability of the blood to carry oxygen (1). When anemia comes on slowly, the symptoms are often vague and may include feeling tired, weakness, shortness of breath or a poor ability to exercise. Anemia that comes on quickly often has greater symptoms, which may include confusion, feeling like one is going to pass out, loss of consciousness, or increased thirst. Anemia must be significant before a person

becomes noticeably pale. Additional symptoms may occur depending on the underlying cause.

The three main types of anemia are due to blood loss, decreased red blood cell production, and increased red blood cell breakdown. Causes of blood loss include trauma and gastrointestinal bleeding, among others. Causes of decreased production include iron deficiency, a lack of vitamin B12, thalassemia, and a number of neoplasms of the bone marrow. Causes of increased breakdown include a number of genetic conditions such as sickle cell anemia, infections like malaria, and certain autoimmune diseases. It can also be classified based on the size of red blood cells and amount of hemoglobin in each cell. If the cells are small, it is microcytic anemia. If they are large, it is macrocytic anemia while if they are normal sized, it is normocytic anemia. Diagnosis in men is based on a hemoglobin of less than 130 to 140 g/L (13 to 14 g/dL), while in women, it must be less than 120 to 130 g/L (12 to 13 g/dL). Further testing is then required to determine the cause.

Anemia due to impaired red blood cells production

Microcytic anemia

Microcytic anaemia is any of several types of anaemia characterized by small red blood cells (called microcytes). The normal mean corpuscular volume (abbreviated to MCV on full blood count results) is 80-100 fL, with smaller cells (<80 fL) described as microcytic and larger cells (>100 fL) as macrocytic (the latter occur in macrocytic anemia).The MCV is the average red blood cell size.

In microcytic anaemia, the red blood cells (erythrocytes) are usually also hypochromic, meaning that the red blood cells appear paler than usual. This is reflected by a lower-than-normal mean corpuscular hemoglobin concentration (MCHC), a measure representing the amount of hemoglobin per unit volume of fluid inside the cell; normally about 320-360 g/L or 32-36 g/dL. Typically, therefore, anemia of this category is described as “microcytic, hypochromic anaemia”.

There are five main causes of microcytic anemia forming the acronym TAILS. Thalassemia, Anemia of chronic disease, Iron deficiency, Lead poisoning and congenital sideroblastic anemia. Only the first three are common in most parts of the world. In theory, these three can be differentiated by their red blood cell (RBC) morphologies.

Iron-deficiency anemia

Iron-deficiency anemia is anemia caused by a lack of iron. Anemia is defined as a decrease in the number of red blood cells or the amount of hemoglobin in the blood (2). When onset is slow, symptoms are often vague such as feeling tired, weak, short of breath, or having decreased ability to exer-

cise. Anemia that comes on quickly often has more severe symptoms, including: confusion, feeling like one is going to pass out or increased thirst. Anemia is typically significant before a person becomes noticeably pale. Children with iron deficiency anemia may have problems with growth and development .There may be additional symptoms depending on the underlying cause.

Epidemiology

Iron-deficiency anemia affected about 1.48 billion people in 2015. A lack of dietary iron is estimated to cause approximately half of all anemia cases globally. Women and young children are most commonly affected. In 2015 anemia due to iron deficiency resulted in about 54,000 deaths – down from 213,000 deaths in 1990.

Mechanism

Anemia can result from significant iron deficiency. When the body has sufficient iron to meet its needs (functional iron), the remainder is stored for later use in cells, mostly in the bone marrow and liver. These stores are called ferritin complexes and are part of the human iron metabolism systems. Men store about 3.5g of iron in their body, and women store about 2.5g (3).

Iron is a mineral that is important in the formation of red blood cells in the body, particularly as a critical component of hemoglobin. About 70% of the iron found in the body is bound to hemoglobin. Iron is primarily absorbed in the small intestine, in particular the duodenum and jejunum. Certain factors increase or decrease absorption of iron. For example, taking Vitamin C with a source of iron is known to increase absorption. Some medications such as tetracyclines and antacids can decrease absorption of iron. After being absorbed in the small intestine, iron travels through blood, bound to transferrin, and eventually ends up in the bone marrow, where it is involved in red blood cell formation. When red blood cells are degraded, the iron is recycled by the body and stored.

When the amount of iron needed by the body exceeds the amount of iron that is readily available, the body can use iron stores (ferritin) for a period of time, and red blood cell formation continues normally. However, as these stores continue to be used, iron is eventually depleted to the point that red blood cell formation is abnormal. Ultimately, anemia ensues, which by definition is a hemoglobin lab value below normal limits.

Causes

It can be caused by increased iron demand, increased iron loss, or decreased iron intake. Increased iron demand often occurs during periods of growth, such as in children and pregnant women. For example, during stages of rapid growth, babies and adolescents may outpace their dietary intake of iron which can result in deficiency in the absence of disease or a grossly abnormal diet. Iron loss is typically from blood loss. One example of blood loss is by chronic gastrointestinal blood loss, which could be

linked to a possible malignancy. In women of childbearing age, heavy menstrual periods can be a source of blood loss causing iron-deficiency anemia. People who do not consume much iron in their diet, such as vegans or vegetarians, are also at increased risk of developing iron deficiency anemia.

Parasitic disease The leading cause of iron-deficiency anemia worldwide is a parasitic disease known as a helminthiasis caused by infestation with parasitic worms (helminths); specifically, hookworms. The hookworms most commonly responsible for causing iron-deficiency anemia include Ancylostoma duodenale, Ancylostoma ceylanicum, and Necator americanus. The World Health Organization estimates that approximately two billion people are infected with soil-transmitted helminths worldwide. Parasitic worms cause both inflammation and chronic blood loss by binding to a human's small-intestinal mucosa, and through their means of feeding and degradation, they can ultimately cause iron-deficiency anemia.

Blood loss Red blood cells contain iron, so blood loss also leads to a loss of iron. There are several causes of blood loss including menstrual bleeding, gastrointestinal bleeding, stomach ulcers, and bleeding disorders. The bleeding may occur quickly or slowly. Slow, chronic blood loss within the body — such as from a peptic ulcer, angiodysplasia, inflammatory bowel disease, a colon polyp or gastrointestinal cancer (e.g., colon cancer)-can cause iron-deficiency anemia.

Menstrual bleeding menstrual bleeding is a common cause of iron deficiency anemia in women of child bearing age. Women with menorrhagia (heavy menstrual periods) are at risk of iron-deficiency anemia because they are at higher-than-normal risk of losing a larger amount blood during menstruation than is replaced in their diet. Most women lose about 40 mL of blood per cycle. Iron is lost with the blood.

Gastrointestinal bleeding the most common cause of iron deficiency anemia in men and post-menopausal women is gastrointestinal bleeding. There are many sources of gastrointestinal tract bleeding including the stomach, esophagus, small intestine, and the large intestine (colon).Gastrointestinal bleeding can result from regular use of some groups of medication, such as NSAIDs (e.g. aspirin), as well as antiplatelets such as clopidogrel and anticoagulants such as warfarin; however, these are required in some patients, especially those with states causing a tendency to form blood clots. Colon cancer is another potential cause gastrointestinal bleeding, thus iron deficiency anemia. Typically colon cancer occurs in older individuals In addition, some bleeding disorders can cause gastrointestinal bleeding. Two examples of bleeding disorders are von Willebrand disease and polycythemia vera.

Diet The body normally gets the iron it requires from foods. If a person consumes too little iron, or iron that is poorly absorbed (non-heme iron), they can become iron defi-

cient over time. Examples of iron-rich foods include meat, eggs, leafy green vegetables and iron-fortified foods. For proper growth and development, infants and children need iron from their diet. For children, a high intake of cow's milk is associated with an increased risk of iron-deficiency anemia. Other risk factors for iron-deficiency anemia include low meat intake and low intake of iron-fortified products.

Iron malabsorption Iron from food is absorbed into the bloodstream in the small intestine, primarily in the duodenum. Iron malabsorption is a less common cause of iron-deficiency anemia, but many gastrointestinal disorders can reduce the body's ability to absorb iron. There are different mechanisms that may be present, such as:In celiac disease, abnormal changes in the structure of the duodenum can decrease iron absorption.Abnormalities or surgical removal of the stomach can also lead to malabsorption by altering the acidic environment needed for iron to be converted into its absorbable form. If there is insufficient production of hydrochloric acid in the stomach, hypochlorhydria/achlorhydria can occur (often due to chronic H. pylori infections or long-term proton pump inhibitor therapy), inhibiting the conversion of ferric iron to the absorbable ferrous iron.

Pregnant women without iron supplementation, iron-deficiency anemia occurs in many pregnant women because their iron stores need to serve their own increased blood volume, as well as be a source of hemoglobin for the growing baby and for placental development. Other less common causes are intravascular hemolysis and hemoglobinuria. Iron deficiency in pregnancy appears to cause long-term and irreversible cognitive problems in the baby.

Children: Babies are at increased risk of developing iron deficiency anemia due to their rapid growth.Their need for iron is greater than they are getting in their diet. Babies are born with iron stores; however, these iron stores typically run out by 4-6 months of age. In addition, infants who are given cow's milk too early can develop anemia due to gastrointestinal blood loss. Children who are at risk for iron-deficiency anemia include: Preterm infants,Low birth weight infants, Infants fed with cow milk under 12 months of age, Breastfed infants who have not received iron supplementation after age 6 months, or those receiving non-iron-fortified formulas, Children between the ages of 1 to 5 years old who receive more than 24 ounces (700 mL) of cow milk per day, Children with low socioeconomic status, Children with special health care needs, Children of Hispanic ethnicity, Children who are overweight.

Blood donation: frequent blood donors are also at risk for developing iron deficiency anemia. When whole blood is donated, approximately 200mg of iron is also lost from the body. The blood bank screens people for anemia before drawing blood for donation. If the patient has anemia, blood is not

drawn. Less iron is lost if the person is donating platelets or white blood cells.

Signs and symptoms

Iron deficiency anemia may be present without a person experiencing symptoms (4). If symptomatic, patients may present with the sign of pallor (reduced oxyhemoglobin in skin or mucous membranes), and the symptoms of fatigue, lightheadedness, decreased exercise tolerance, headache, and weakness. None of these symptoms (or any of the others below) are sensitive or specific. The symptom most suggestive of iron deficiency anemia in children is pallor of mucous membranes (primarily the conjunctiva). Even so, a large study showed that pallor of the mucous membranes is only 28% sensitive and 87% specific (with high predictive value) in distinguishing children with anemia (defined as hemoglobin < 11.0 g/dl) and 49% sensitive and 79% specific in distinguishing severe anemia (hemoglobin < 7.0 g/dl). Thus, this sign is reasonably predictive when present, but not helpful when absent, as only one-third to one-half of children who are anemic (depending on severity) will show pallor.

Iron deficiency anemia tends to develop slowly; therefore the body has time to adapt, and the disease often goes unrecognized for some time.In severe cases, shortness of breath can occur. Pica may also develop; of which consumption of ice, known as pagophagia, has been suggested to be the most specific for iron deficiency anemia.

Other possible symptoms and signs of iron-deficiency anemia include:

-Koilonychia (spoon-shaped nails).

-Irritability.

-Angina (chest pain).

-Palpitations (feeling that the heart is skipping beats or fluttering).

-Breathlessness.

-Tingling, numbness, or burning sensations.

-Glossitis (inflammation or infection of the tongue).

-Angular cheilitis (inflammatory lesions at the mouth's corners).

-Koilonychia (spoon-shaped nails) or nails that are brittle.

-Poor appetite.

-Dysphagia (difficulty swallowing) due to formation of esophageal webs (Plummer-Vinson syndrome).

-Restless legs syndrome.

-Child development, Iron-deficiency anemia is associated with poor neurological development, including decreased learning ability and altered motor functions. This is because iron deficiency impacts the development of the cells of the brain called neurons. When the body is low on iron, the red blood cells get priority on iron and it is shifted away from the neurons of the brain. Exact causation has not been established, but

there is a possible long-term impact from these neurological issues.

Diagnosis

Blood smear of a person with iron-deficiency anemia at 40X enhancement Conventionally, a definitive diagnosis requires a demonstration of depleted body iron stores obtained by bone marrow aspiration, with the marrow stained for iron.However, with the availability of reliable blood tests that can be more readily collected for iron-deficiency anemia diagnosis, a bone marrow aspiration is usually not obtained.

A thorough medical history is important to the diagnosis of iron-deficiency anemia. The history can help to differentiate common causes of the condition such as a menstruation in woman or the presence of blood in the stool. A travel history to areas in which hookworms and whipworms are endemic may also be helpful in guiding certain stool tests for parasites or their eggs. Although symptoms can play a role in identifying iron-deficiency anemia, they are often Vague, which may limit their contribution to determining the diagnosis.

Blood tests

Change in lab values in iron deficiency anemia,Change Parameter:

↓Ferritin, hemoglobin, MCV, MCH

↑TIBC, transferrin, RDW, FEP (Free erythrocyte protoporphyrin)

Anemia is often discovered by routine blood tests. A sufficiently low hemoglobin (Hb) by definition makes the diagnosis of anemia, and a low hematocrit value is also characteristic of anemia. Further studies will be undertaken to determine the anemia's cause. If the anemia is due to iron deficiency, one of the first abnormal values to be noted on a CBC, as the body's iron stores begin to be depleted, will be a high red blood cell distribution width (RDW), reflecting an increased variability in the size of red blood cells (RBCs). A low mean corpuscular volume (MCV) also appears during the course of body iron depletion. It indicates a high number of abnormally small red blood cells. A low MCV, a low mean corpuscular hemoglobin or mean corpuscular hemoglobin concentration (MCH), and the corresponding appearance of RBCs on visual examination of a peripheral blood smear narrows the problem to a microcytic anemia (literally, a small red blood cell anemia).

The blood smear of a person with iron-deficiency anemia shows many hypochromic (pale, relatively colorless) and small RBCs, and May also show poikilocytosis (variation in shape) and anisocytosis (variation in size). Target cells may also be seen. With more severe iron-deficiency anemia, the peripheral blood smear may show hypochromic, pencil-shaped cells and, occasionally, small numbers of nucleated red blood cells. The platelet count may be slightly above the high limit of normal in iron-deficiency anemia (termed a mild thrombocytosis), but severe

cases can present with thrombocytopenia (low platelet count).

Iron-deficiency anemia is confirmed by tests that include serum ferritin, serum iron level, serum transferrin, and total iron binding capacity (TIBC). A low serum ferritin is most commonly found. However, serum ferritin can be elevated by any type of chronic inflammation and thus is not consistently decreased in iron-deficiency anemia. Serum iron levels may be measured, but serum iron concentration is not as reliable as the measurement of both serum iron and serum iron-binding protein levels (TIBC). The ratio of serum iron to TIBC (called iron saturation or transferrin saturation index or percent) is a value with defined parameters that can help to confirm the diagnosis of iron-deficiency anemia; however, other conditions must also be considered, including other types of anemia.

Another finding that can be used is the level of free erythrocyte protoporphyrin (FEP). During haemoglobin synthesis, trace amounts of zinc will be incorporated into protoporphyrin in the place of iron which is lacking. We can separate the protoporphyrin from its zinc moeity and measure it, known as the FEP, providing an indirect measurement of the zinc-protoporphyrin complex. The level of FEP is expressed in either µg/dl of whole blood or µg/dl of RBC. An iron insufficiency in the bone marrow can be detected very early by a rise in FEP.

Further testing may be necessary to differentiate iron-deficiency anemia from other disorders, such as thalassemia minor. It is very important not to treat people with thalassemia with an iron supplement, as this can lead to hemochromatosis. A hemoglobin electrophoresis provides useful evidence for distinguishing these two conditions, along with iron studies.

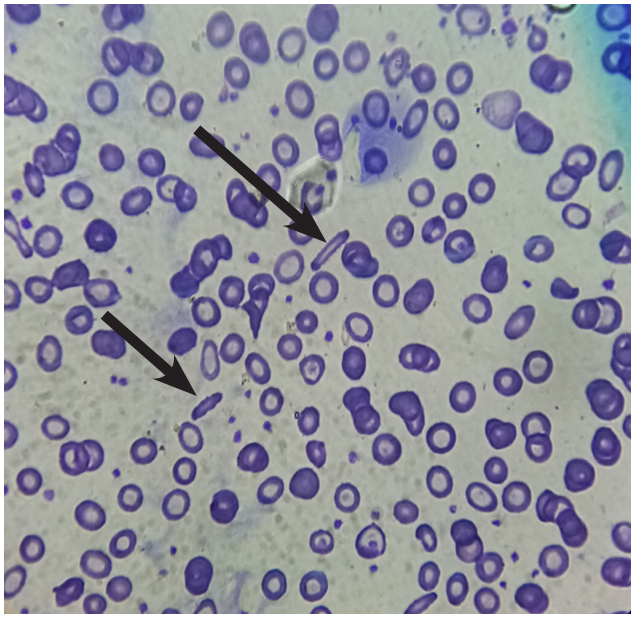


Figure (3-1) Blood film from IDA patient stained by Romanowsky stain shows microcytic hypochromic cells with many pencil cells.

Treatment

Treatment should take into account the cause and severity of the condition. If the iron-deficiency anemia is a result of blood loss or another underlying cause, treatment is geared toward addressing the underlying cause. Most cases of iron deficiency anemia are treated with oral iron supplements. In severe acute cases, treatment measures are taken for immediate management in the interim, such as blood transfusions or intravenous iron.

For less severe cases, treatment of iron-deficiency anemia includes dietary changes to incorporate iron-rich foods into regular oral intake and oral iron supplementation. Foods rich in ascorbic acid (vitamin C) can also be beneficial, since ascorbic acid enhances iron absorption. Oral iron supplements are available in multiple forms. Some are in the form of pills and some are drops for children. Most forms of oral iron replacement therapy are absorbed well by the small intestine; however, there are certain preparations of iron supplements that are designed for longer release in the small intestine than other preparations. Oral iron supplements are best taken up by the body on an empty stomach because food can decrease the amount of iron absorbed from the small intestine. The dosing of oral iron replacement therapy is as much as 200 mg per day. This is generally spread out as 3-4 pills taken throughout the day. The various forms of treatment are not without possible adverse side effects. Iron supplementation by mouth commonly causes negative gastrointestinal effects, including constipation. Constipation is reported by 15-20% of patients taking oral iron therapy. Preparations of iron therapy that take longer to be absorbed by the small intestine (extended release iron therapy) are less likely to cause constipation. It can take six months to one year to get blood levels of iron up to a normal range and provide the body with iron stores.

As iron-deficiency anemia becomes more severe, if the anemia does not respond to oral treatments, or if the treated person does not tolerate oral iron supplementation, then other measures may become necessary. Two options are intravenous iron injections and blood transfusion. Intravenous can be for people who do not tolerate oral iron or who require iron on a long term basis. For example, people receiving dialysis treatment who are also getting erythropoietin or another erythropoiesis-stimulating agent are given parenteral iron, which helps the body respond to the erythropoietin agents to produce red blood cells.

Intravenous iron can induce an allergic response that can be as serious as anaphylaxis, although different formulations have decreased the likelihood of this adverse effect. In certain cases intravenous iron is both safer and more effective than the oral route. For patients with severe anemia such as from blood loss, or who have severe symptoms such as cardiovascular instability, a blood transfusion may be considered.

Prevention

Iron deficiency anemia can be prevented by eating a diet containing sufficient amounts of iron or by iron supplementation. Foods high in iron include meat, nuts, spinach, and foods made with iron-fortified flour. Treatment may include dietary changes and dealing with underlying causes.

Anemia of chronic disease

Anemia of chronic disease, or anemia of

chronic inflammation, is a form of anemia seen in chronic infection, chronic immune activation, and malignancy. These conditions all produce massive elevation of Interleukin-6, which stimulates hepcidin production and release from the liver, which in turn reduces the iron carrier protein ferroportin so that access of iron to the circulation is reduced. Other mechanisms may also play a role, such as reduced erythropoiesis.

Mechanism

In response to inflammatory cytokines, increasingly IL-6, the liver produces increased amounts of hepcidin. Hepcidin in turn causes increased internalisation of ferroportin molecules on cell membranes which prevents release from iron stores. Inflammatory cytokines also appear to affect other important elements of iron metabolism, including decreasing ferroportin expression, and probably directly blunting erythropoiesis by decreasing the ability of the bone marrow to respond to erythropoietin.

Before the recent discovery of hepcidin and its function in iron metabolism, anemia of chronic disease was seen as the result of a complex web of inflammatory changes. Over the last few years, however, many investigators have come to feel that hepcidin is the central actor in producing anemia of chronic inflammation.

In addition to effects of iron sequestration, inflammatory cytokines promote the production of white blood cells. Bone marrow produces both white blood cells and red blood cells from the same precursor stem cells. Therefore, the upregulation of white blood cells causes fewer stem cells to differentiate into red blood cells. This effect may be an important additional cause for the decreased erythropoiesis and red blood cell production seen in anemia of inflammation, even when erythropoietin levels are normal, and even aside from the effects of hepcidin. Nonetheless, there are other mechanisms that also contribute to the lowering of hemoglobin levels during inflammation:

(i) Inflammatory cytokines suppress the proliferation of erythroid precursors in the bone marrow.

(ii) Inflammatory cytokines inhibit the release of erythropoietin (EPO) from the kidney.

(iii) The survival of circulating red cells is shortened.

In the short term, the overall effect of these changes is likely positive: it allows the body to keep more iron away from bacterial pathogens in the body, while producing more immune cells to fight off infection. Almost all bacteria depend on iron to live and multiply. However, if inflammation continues, the effect of locking up iron stores is to reduce the ability of the bone marrow to produce red blood cells. These cells require iron for their massive amounts of hemoglobin which allow them to transport oxygen.

Because anemia of chronic disease can be the result of non-infective causes of inflammation, future research is likely to investigate whether hepcidin antagonists might be able to treat this problem.

Anemia of chronic disease may also be due to neoplastic disorders and non-infectious inflammatory diseases. Neoplastic disorders include Hodgkin's disease lung and breast carcinoma and non-infectious inflammatory diseases include rheumatoid arthritis and systemic lupus erythematosus.

Anemia of chronic disease as it is now understood is to at least some degree separate from the anemia seen in renal failure in which anemia results from poor production of erythropoietin, or the anemia caused by some drugs (like AZT, used to treat HIV infection) that have the side effect of inhibiting erythropoiesis. In other words, not all anemia seen in people with chronic disease should be diagnosed as anemia of chronic disease. On the other hand, both of these examples show the complexity of this diagnosis: HIV infection itself can produce anemia of chronic disease, and renal failure can lead to inflammatory changes that also can produce anemia of chronic disease.

Survival advantage

Limiting some microbes' access to iron can reduce their virulence, thereby potentially reducing the severity of infection. Blood transfusion to patients with anemia of chronic disease is associated with a higher mortality, supporting the concept.

Severity Anemia of chronic disease is usually mild but can be severe. It is usually normocytic, but can be microcytic.The presence of both anemia of chronic disease and dietary iron deficiency in the same patient results in a more severe anemia.

Diagnosis

While no single test is reliable to distinguish iron deficiency anemia from the anemia of chronic inflammation, there are sometimes some suggestive data:

In anemia of chronic inflammation without iron deficiency, ferritin is normal or high, reflecting the fact that iron is sequestered within cells, and ferritin is being produced as an acute phase reactant. In iron deficiency anemia ferritin is low. Total iron-binding capacity (TIBC) is high in iron deficiency, reflecting production of more transferrin to increase iron binding; TIBC is low or normal in anemia of chronic inflammation.

Treatment

The ideal treatment for anemia of chronic disease is to treat the chronic disease successfully, but this is rarely possible.

Parenteral iron is increasingly used for anemia in chronic renal disease and inflammatory bowel disease. Erythropoietin can be helpful, but this is costly and may be dangerous. Erythropoietin is advised either in conjunction with adequate iron replacement which in practice is intravenous, or when IV

iron has proved ineffective.

Aplastic anemia

Aplastic anaemia is a rare disease in which the bone marrow and the hematopoietic stem cells that reside there are damaged. This causes a deficiency of all three blood cell types (pancytopenia): red blood cells (anemia), white blood cells (leukopenia), and platelets (thrombocytopenia). Aplastic refers to the inability of stem cells to generate mature blood cells.

It is more frequent in people in their teens and twenties, but is also common among the elderly. It can be caused by heredity, immune disease, or exposure to chemicals, drugs, or radiation. However, in about half the cases, the cause is unknown.

Signs and symptoms

Anemia may lead to feeling tired, pale skin and a fast heartbeat. Low platelets are associated with an increased risk of bleeding, bruising and petechiae. Low white blood cells increase the risk of infections.

Causes

Aplastic anemia can be caused by exposure to certain chemicals, drugs, radiation, infection, immune disease; in about half the cases, a definitive cause is unknown. It is not a familial line hereditary condition, nor is it contagious. It can be acquired due to exposure to other conditions but if a person develops the condition, their offspring would not develop it by virtue of their genetic relationship.

Aplastic anemia is also sometimes associated with exposure to toxins such as benzene, or with the use of certain drugs, including chloramphenicol, carbamazepine, felbamate, phenytoin, quinine, and phenylbutazone. Many drugs are associated with aplasia mainly according to case reports, but at a very low probability. As an example, chloramphenicol treatment associated with aplasia in less than one in 40,000 treatment courses, and carbamazepine aplasia is even rarer.

Exposure to ionizing radiation from radioactive materials or radiation-producing devices is also associated with the development of aplastic anemia. Marie Curie, famous for her pioneering work in the field of radioactivity, died of aplastic anemia after working unprotected with radioactive materials for a long period of time; the damaging effects of ionizing radiation were not then known.

One known cause is an autoimmune disorder in which white blood cells attack the bone marrow.

Short-lived aplastic anemia can also be a result of parvovirus infection. In humans, the P antigen (also known as globoside), one of the many cellular receptors that contribute to a person's blood type, is the cellular receptor for parvovirus B19 virus that causes erythema infectiosum (fifth disease) in children. Because it infects red blood cells

as a result of the affinity for the P antigen, parvovirus causes complete cessation of red blood cell production. In most cases, this goes unnoticed, as red blood cells live on average 120 days, and the drop in production does not significantly affect the total number of circulating red blood cells. In people with conditions where the cells die early (such as sickle cell disease), however, parvovirus infection can lead to severe anemia.

More frequently parvovirus B19 is associated with aplastic crisis which involves only the red blood cells (despite the name). Aplastic anemia involves all different cell lines.

Viruses that have been linked to the development of aplastic anemia include hepatitis, Epstein-Barr, cytomegalovirus, parvovirus B19 and HIV.

Diagnosis

The condition needs to be differentiated from pure red cell aplasia. In aplastic anemia, the patient has pancytopenia (i.e., leukopenia and thrombocytopenia) resulting in decrease of all formed elements. In contrast, pure red cell aplasia is characterized by reduction in red cells only. The diagnosis can only be confirmed on bone marrow examination. Before this procedure is undertaken, a patient will generally have had other blood tests to find diagnostic clues, including a complete blood count, renal function and electrolytes, liver enzymes, thyroid function tests, vitamin B12 and folic acid levels.

The following tests aid in determining differential diagnosis for aplastic anemia:

Bone marrow aspirate and biospy: to rule out other causes of pancytopenia (i.e. neoplastic infiltration or significant myelofibrosis).

History of iatrogenic exposure to cytotoxic chemotherapy: can cause transient bone marrow suppression

X-rays, computed tomography (CT) scans, or ultrasound imaging tests: enlarged lymph nodes (sign of lymphoma), kidneys and bones in arms and hands (abnormal in Fanconi anemia)

Chest X-ray: infections

Liver tests: liver diseases

Viral studies: viral infections

Vitamin B12 and folate levels: vitamin deficiency

Blood tests for paroxysmal nocturnal hemoglobinuria

Test for antibodies: immune competency

Treatment

Treating immune-mediated aplastic anemia involves suppression of the immune system, an effect achieved by daily medicine intake, or, in more severe cases, a bone marrow transplant, a potential cure. The transplanted bone marrow replaces the failing bone marrow cells with new ones from a matching donor. The multipotent stem cells in the bone marrow reconstitute all three

blood cell lines, giving the patient a new immune system, red blood cells, and platelets. However, besides the risk of graft failure, there is also a risk that the newly created white blood cells may attack the rest of the body ("graft-versus-host disease"). In young patients with an HLA matched sibling donor, bone marrow transplant can be considered as first-line treatment, patients lacking a matched sibling donor typically pursue immunosuppression as a first-line treatment, and matched unrelated donor transplants are considered a second-line therapy.

Medical therapy of aplastic anemia often includes a course of antithymocyte globulin (ATG) and several months of treatment with cyclosporine to modulate the immune system. Chemotherapy with agents such as cyclophosphamide may also be effective but has more toxicity than ATG. Antibody therapy, such as ATG, targets T-cells, which are believed to attack the bone marrow. Corticosteroids are generally ineffective, though they are used to ameliorate serum sickness caused by ATG. Normally, success is judged by bone marrow biopsy 6 months after initial treatment with ATG.

One prospective study involving cyclophosphamide was terminated early due to a high incidence of mortality, due to severe infections as a result of prolonged neutropenia.

In the past, before the above treatments became available, patients with low leukocyte counts were often confined to a sterile room or bubble (to reduce risk of infections), as in the case of Ted DeVita.

Follow-up

Full blood counts are required on a regular basis to determine whether the patient is still in a state of remission.

Many patients with aplastic anemia also have clones of cells characteristic of the rare disease paroxysmal nocturnal hemoglobinuria (PNH, anemia with thrombopenia and/or thrombosis), sometimes referred to as AA/PNH. Occasionally PNH dominates over time, with the major manifestation intravascular hemolysis. The overlap of AA and PNH has been speculated to be an escape mechanism by the bone marrow against destruction by the immune system. Flow cytometry testing is performed regularly in people with previous aplastic anemia to monitor for the development of PNH.

Prognosis

Untreated, severe aplastic anemia has a high risk of death. Modern treatment, by drugs or stem cell transplant, has a five-year survival rate that exceeds 85%, with younger age associated with higher survival.

Survival rates for stem cell transplant vary depending on age and availability of a well-matched donor. Five-year survival rates for patients who receive transplants have been shown to be 82% for patients under age 20, 72% for those 20–40 years old, and closer to 50% for patients over age 40. Success rates are better for patients who have donors that

are matched siblings and worse for patients who receive their marrow from unrelated donors.

Older people (who are generally too frail to undergo bone marrow transplants), and people who are unable to find a good bone marrow match, undergoing immune suppression have five-year survival rates of up to 75%.

Relapses are common. Relapse following ATG/cyclosporin use can sometimes be treated with a repeated course of therapy. In addition, 10-15% of severe aplastic anemia cases evolve into myelodysplastic syndrome and leukemia. According to a study, for children who underwent immunosuppressive therapy, about 15.9% of children who responded to immunosuppressive therapy encountered relapse.

Milder disease can resolve on its own.

Sideroblastic anemia

Sideroblastic anemia or sideroachrestic anemia is a form of anemia in which the bone marrow produces ringed sideroblasts rather than healthy red blood cells (erythrocytes).

In sideroblastic anemia, the body has iron available but cannot incorporate it into hemoglobin, which red blood cells need in order to transport oxygen efficiently. The disorder may be caused either by a genetic disorder or indirectly as part of myelodysplastic syndrome, which can develop into hematological malignancies (especially acute myeloid leukemia).

Sideroblasts

Sideroblasts (sidero- + -blast) are nucleated erythroblasts (precursors to mature red blood cells) with granules of iron accumulated in the mitochondria surrounding the nucleus. Normally, sideroblasts are present in the bone marrow, and enter the circulation after maturing into a normal erythrocyte. The presence of sideroblasts does not define sideroblastic anemia. Only the finding of ring (or ringed) sideroblasts characterizes sideroblastic anemia.

Ring sideroblasts are named so because iron-laden mitochondria form a ring around the nucleus. It is a subtype of basophilic granules of the erythrocyte, but which can only be seen in bone marrow. To count a cell as a ring sideroblast, the ring must encircle a third or more of the nucleus and contain five or more iron granules.

The WHO International Working Group on Morphology of MDS (IWGM-MDS) defined three types of sideroblasts:

Type 1 sideroblasts: fewer than 5 siderotic granules in the cytoplasm

Type 2 sideroblasts: 5 or more siderotic granules, but not in a perinuclear distribution

Type 3 or ring sideroblasts: 5 or more granules in a perinuclear position, surrounding the nucleus or encompassing at least one third of the nuclear circumference.

Type 1 and type 2 are found in Non-sideroblastic anemias. Type 3 is found only in Sideroblastic anemia.

Classification

Sideroblastic anemia is typically divided into subtypes based on its cause.

*Hereditary or congenital sideroblastic anemia may be X-linked or autosomal.

*Acquired, or secondary, sideroblastic anemia develops after birth and is divided according to its cause.

Symptoms

Symptoms of sideroblastic anemia include skin paleness, fatigue, dizziness, and enlarged spleen and liver. Heart disease, liver damage, and kidney failure can result from iron buildup in these organs.

Causes

Causes of sideroblastic anemia can be categorized into three groups: congenital sideroblastic anemia, acquired clonal sideroblastic anemia, and acquired reversible sideroblastic anemia. All cases involve dysfunctional heme synthesis or processing. This leads to granular deposition of iron in the mitochondria that form a ring around the nucleus of the developing red blood cell. Congenital forms often present with normocytic or microcytic anemia while acquired forms of sideroblastic anemia are often normocytic or macrocytic.

Congenital Sideroblastic Anemia

X-linked sideroblastic anemia: This is the most common congenital cause of sideroblastic anemia and involves a defect in ALAS2, which is involved in the first step of heme synthesis. Although X-linked, approximately one third of patients are women due to skewed X-inactivation (lyonizations).

Autosomal recessive sideroblastic anemia involves mutations in the SLC25A38 gene. The function of this protein is not fully understood, but it is involved in mitochondrial transport of glycine. Glycine is a substrate for ALAS2 and necessary for heme synthesis. The autosomal recessive form is typically severe in presentation.

Genetic syndromes: Rarely, sideroblastic anemia may be part of a congenital syndrome and present with associated findings, such as ataxia, myopathy, and pancreatic insufficiency.

Acquired Clonal Sideroblastic Anemia

Clonal sideroblastic anemias fall under the broader category of myelodysplastic syndromes (MDS). Three forms exist and include refractory anemia with ringed sideroblasts (RARS), refractory anemia with ringed sideroblasts and thrombocytosis (RARS-T), and refractory cytopenia with multilineage dysplasia and ringed sideroblasts (RCMD-RS). These anemias are associated with increased risk for leukemic evolution.

Acquired reversible sideroblastic anemia

Causes include excessive alcohol use (the most common cause of sideroblastic anemia), pyridoxine deficiency (vitamin B6 is the cofactor in the first step of heme synthesis), lead poisoning and copper deficiency. Excess zinc can indirectly cause sideroblastic anemia by decreasing absorption and increasing excretion of copper. Antimicrobials that may lead to sideroblastic anemia include isoniazid (which interferes with pyridoxine metabolism), chloramphenicol (which, by inhibiting the synthesis of mitochondrial membrane protein, impairs mitochondrial respiration), cycloserine, and linezolid.

Diagnosis

Ringed sideroblasts are seen in the bone marrow. On the peripheral blood smear can be found erythrocytes with basophilic stippling (cytoplasmic granules of RNA precipitates) and Pappenheimer bodies (cytoplasmic granules of iron).

The anemia is moderate to severe and dimorphic. Microscopic viewing of the red blood cells will reveal marked unequal cell size and abnormal cell shape. Basophilic stippling is marked and target cells are common. The mean cell volume is commonly decreased (i.e., a microcytic anemia), but it may also be normal or even high. The RDW is increased with the red blood cell histogram shifted to the left. Leukocytes and platelets are normal. Bone marrow shows erythroid hyperplasia with a maturation arrest.

In excess of 40% of the developing erythrocytes are ringed sideroblasts. Serum iron, percentage saturation and ferritin are increased. The total iron-binding capacity of the cells is normal to decreased. Stainable marrow hemosiderin is increased.

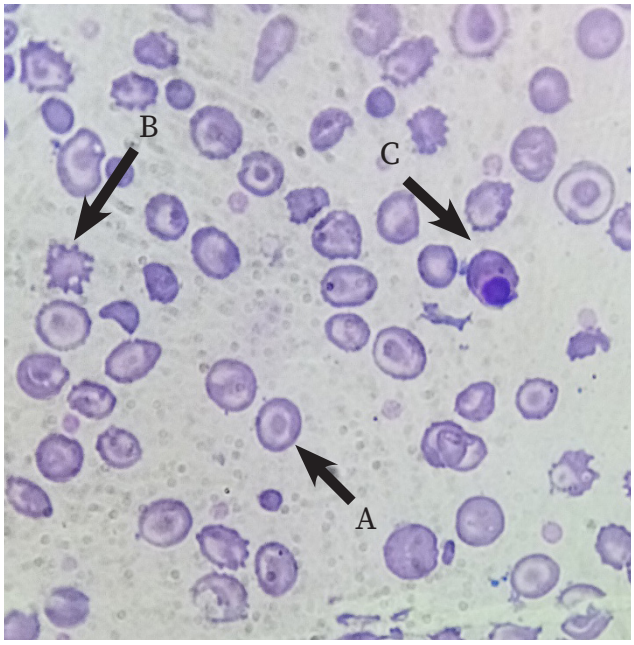


Figure (3-2) A: target cells, B: crenated cells (Echinocytes), C: nucleated RBC.

Laboratory findings

Serum Iron: high

Increased ferritin levels

Decreased total iron-binding capacity

High transferrin saturation

Hematocrit of about 20-30%

The mean corpuscular volume or MCV is usually normal or low for congenital causes of sideroblastic anemia but normal or high for acquired forms.

With lead poisoning, see coarse basophilic stippling of red blood cells on peripheral

blood smear

Specific test: Prussian blue stain of RBC in marrow shows ringed sideroblasts. Prussian blue staining involves a non-enzymatic reaction of ferrous iron with ferrocyanide forming ferric-ferrocyanide, which is blue in color. A counterstain may be used to provide better visualization.

Treatment

Occasionally, the anemia is so severe that support with transfusion is required. These patients usually do not respond to erythropoietin therapy. Some cases have been reported that the anemia is reversed or heme level is improved through use of moderate to high doses of pyrodoxine (vitamin B6). In severe cases of SBA, bone marrow transplant is also an option with limited information about the success rate. Some cases are listed on MedLine and various other medical sites. In the case of isoniazid-induced sideroblastic anemia, the addition of B6 is sufficient to correct the anemia. Desferrioxamine, a chelating agent, is used to treat iron overload from transfusions. Therapeutic phlebotomy can be used to manage iron overload.

Prognosis

Sideroblastic anemias are often described as responsive or non-responsive in terms of increased hemoglobin levels to pharmacological doses of vitamin B6.

1- Congenital: 80% are responsive, though the anemia does not completely resolve.

2- Acquired clonal: 40% are responsive, but the response may be minimal.

3- Acquired reversible: 60% are responsive, but course depends on treatment of the underlying cause.

Severe refractory sideroblastic anemias requiring regular transfusions and/or that undergo leukemic transformation (5-10%) significantly reduce life expectancy.

Lead poisoning

Lead poisoning is a type of metal poisoning caused by lead in the body. The brain is the most sensitive (5). Symptoms may include abdominal pain, constipation, headaches, irritability, memory problems, inability to have children, and tingling in the hands and feet. It causes almost 10% of intellectual disability of otherwise unknown cause and can result in behavioral problems. Some of the effects are permanent. In severe cases anemia, seizures, coma, or death may occur.

Exposure to lead can occur by contaminated air, water, dust, food, or consumer products. Children are at greater risk as they are more likely to put objects in their mouth such as those that contain lead paint and absorb a greater proportion of the lead that they eat. Exposure at work is a common cause of lead poisoning in adults with certain occupations at particular risk. Diagnosis is typically by measurement of the blood lead level. The Centers for Disease Control (US) has set the upper limit for blood lead for adults at

10 µg/dl (10 µg/100 g) and for children at 5 µg/dl. Elevated lead may also be detected by changes in red blood cells or dense lines in the bones of children as seen on X-ray.

Prevention and epidemiology

Lead poisoning is preventable. This includes individual efforts such as removing lead-containing items from the home, workplace efforts such as improved ventilation and monitoring, and nationwide policies such as laws that ban lead in products such as paint and gasoline, reduce allowable levels in water or soil, and provide for clean-up of contaminated soil. The major treatments are removal of the source of lead and the use of medications that bind lead so it can be eliminated from the body, known as chelation therapy. Chelation therapy in children is recommended when blood levels are greater than 40–45 µg/dl. Medications used include dimercaprol, edetate calcium disodium, and succimer.

In 2016 lead is believed to have resulted in 540,000 deaths. It occurs most commonly in the developing world. Those who are poor are at greater risk. Lead is believed to result in 0.6% of the world's disease burden. People have been mining and using lead for thousands of years. Descriptions of lead poisoning date to at least 2000 BC, while efforts to limit leads use date back to at least the 16th century. Concerns for low levels of exposure begin in the 1970s with there being no safe threshold for lead exposure.

Classification

Classically, "lead poisoning" or "lead intoxication" has been defined as exposure to high levels of lead typically associated with severe health effects. Poisoning is a pattern of symptoms that occur with toxic effects from mid to high levels of exposure; toxicity is a wider spectrum of effects, including subclinical ones (those that do not cause symptoms). However, professionals often use "lead poisoning" and "lead toxicity" interchangeably, and official sources do not always restrict the use of "lead poisoning" to refer only to symptomatic effects of lead.

The amount of lead in the blood and tissues, as well as the time course of exposure, determine toxicity. Lead poisoning may be acute (from intense exposure of short duration) or chronic (from repeat low-level exposure over a prolonged period), but the latter is much more common. Diagnosis and treatment of lead exposure are based on blood lead level (the amount of lead in the blood), measured in micrograms of lead per deciliter of blood (µg/dL). Urine lead levels may be used as well, though less commonly. In cases of chronic exposure lead often sequesters in the highest concentrations first in the bones, then in the kidneys. If a provider is performing a provocative excretion test, or "chelation challenge", a measurement obtained from urine rather than blood is likely to provide a more accurate representation of total lead burden to a skilled interpreter.

The US Centers for Disease Control and Pre-

vention and the World Health Organization state that a blood lead level of 10 µg/dL or above is a cause for concern; however, lead may impair development and have harmful health effects even at lower levels, and there is no known safe exposure level. Authorities such as the American Academy of Pediatrics define lead poisoning as blood lead levels higher than 10 µg/dL.

Lead forms a variety of compounds and exists in the environment in various forms. Features of poisoning differ depending on whether the agent is an organic compound (one that contains carbon), or an inorganic one. Organic lead poisoning is now very rare, because countries across the world have phased out the use of organic lead compounds as gasoline additives, but such compounds are still used in industrial settings. Organic lead compounds, which cross the skin and respiratory tract easily, affect the central nervous system predominantly.

Macrocytic anemia

The term macrocytic is from Greek words meaning "large cell". A macrocytic class of anemia is an anemia in which the red blood cells (erythrocytes) are larger than their normal volume. The normal erythrocyte volume in humans is about 80 to 100 femtoliters (fL= 10−15 L). In metric terms the size is given in equivalent cubic micrometers (1 µm3 = 1 fL). The condition of having erythrocytes which (on average) are too large, is called macrocytosis. In contrast, in microcytic anemia, the erythrocytes are smaller than normal.

In a macrocytic anemia, the larger red cells are always associated with insufficient numbers of cells and often also insufficient hemoglobin content per cell. Both of these factors work to the opposite effect of larger cell size, to finally result in a total blood hemoglobin concentration that is less than normal (i.e., anemia).

Megaloblastic anemia

Megaloblastic anemia (or megaloblastic anaemia) is an anemia (of macrocytic classification) that results from inhibition of DNA synthesis during red blood cell production. When DNA synthesis is impaired, the cell cycle cannot progress from the G2 growth stage to the mitosis (M) stage. This leads to continuing cell growth without division, which presents as macrocytosis. Megaloblastic anemia has a rather slow onset, especially when compared to that of other anemias. The defect in red cell DNA synthesis is most often due to hypovitaminosis, specifically vitamin B12 deficiency or folate deficiency. Loss of micronutrients may also be a cause. Copper deficiency resulting from an excess of zinc from unusually high oral consumption of zinc-containing denture-fixation creams has been found to be a cause.

Megaloblastic anemia not due to hypovitaminosis may be caused by antimetabolites that poison DNA production directly, such as some chemotherapeutic or antimicrobial agents (for example azathioprine or tri-

methoprim).

The pathological state of megaloblastosis is characterized by many large immature and dysfunctional red blood cells (megaloblasts) in the bone marrow and also by hypersegmented neutrophils (defined as the presence of neutrophils with six or more lobes or the presence of more than 3% of neutrophils with at least five lobes). These hypersegmented neutrophils can be detected in the peripheral blood.

Causes

(i) Vitamin B12 deficiency:

1. Achlorhydria-induced malabsorption.

2. Deficient intake.

3. Deficient intrinsic factor, a molecule produced by cells in the stomach that is required for B12 absorption (pernicious anemia or gastrectomy).

4. Coeliac disease.

5. Biological competition for vitamin B12 by diverticulosis, fistula, intestinal anastomosis, or infection by the marine parasite Diphyllobothrium latum (fish tapeworm).

6. Selective vitamin B12 malabsorption (congenital—juvenile megaloblastic anemia 1—and drug-induced).

7. Chronic pancreatitis.

8. Ileal resection and bypass.

9. Nitrous oxide anesthesia (usually requires repeated instances).

(ii) Folate deficiency:

1. Alcoholism

2. Deficient intake

3. Increased needs: pregnancy, infant, rapid cellular proliferation, and cirrhosis

4. Malabsorption (congenital and drug-induced)

5. Intestinal and jejunal resection

6. (Indirect) Deficient thiamine and factors (e.g., enzymes) responsible for folate metabolism.

(iii) Combined Deficiency: vitamin B12 & folate:

1. Inherited Pyrimidine Synthesis Disorders: Orotic aciduria

2. Inherited DNA Synthesis Disorders

(iv)Toxins, Drugs and other causes:

1. Folic acid antagonists (methotrexate)

2. Purine synthesis antagonists (6-mercaptopurine)

3. Pyrimidine antagonists (cytarabine)

4. Phenytoin

5. Nitrous Oxide

6. Erythroleukemia

7. Inborn genetic mutations of the Methionine synthase gene

8. Di Guglielmo's syndrome

9. Congenital dyserythropoietic anemia

Diagnosis

Hematological findings: the blood film can point towards vitamin deficiency:

Decreased red blood cell (RBC) count and hemoglobin levels, Increased mean corpuscular volume (MCV, >100 fL) and mean corpuscular hemoglobin (MCH), Normal mean corpuscular hemoglobin concentration (MCHC, 32–36 g/dL)

Decreased reticulocyte count due to destruction of fragile and abnormal megaloblastic erythroid precursor. The platelet count may be reduced. Neutrophil granulocytes may show multisegmented nuclei ("senile neutrophil"). This is thought to be due to decreased production and a compensatory prolonged lifespan for circulating neutrophils, which increase numbers of nuclear segments with age.

Anisocytosis (increased variation in RBC size) and poikilocytosis (abnormally shaped RBCs).

Macrocytes (larger than normal RBCs) are present.

Ovalocytes (oval-shaped RBCs) are present.

Howell-Jolly bodies (chromosomal remnant) also present.

Blood chemistries will also show: An increased lactic acid dehydrogenase (LDH) level. The isozyme is LDH-2 which is typical of the serum and hematopoetic cells.

Increased homocysteine and methylmalonic acid in Vitamin B12 deficiency. Increased homocysteine in folate deficiency

Normal levels of both methylmalonic acid and total homocysteine rule out clinically significant cobalamin deficiency with virtual certainty.

Bone marrow (not normally checked in a patient suspected of megaloblastic anemia) shows megaloblastic hyperplasia.

The gold standard for the diagnosis of Vitamin B12 deficiency is a low blood level of Vitamin B12. A low level of blood Vitamin B12 is a finding that normally can and should be treated by injections, supplementation, or dietary or lifestyle advice, but it is not a diagnosis. Hypovitaminosis B12 can result from a number of mechanisms, including those listed above. For determination of cause, further patient history, testing, and empirical therapy may be clinically indicated.

A measurement of methylmalonic acid (methylmalonate) can provide an indirect method for partially differentiating Vitamin B12 and folate deficiencies. The level of methylmalonic acid is not elevated in folic acid deficiency. Direct measurement of blood cobalamin remains the gold standard because the test for elevated methylmalonic acid is not specific enough. Vitamin B12 is one necessary prosthetic group to the enzyme methylmalonyl-coenzyme A

mutase. Vitamin B12 deficiency is but one among the conditions that can lead to dysfunction of this enzyme and a buildup of its substrate, methylmalonic acid, the elevated level of which can be detected in the urine and blood.

Due to the lack of available radioactive Vitamin B12, the Schilling test is now largely a historical artifact. The Schilling test was performed in the past to help determine the nature of the vitamin B12 deficiency. An advantage of the Schilling test was that it often included Vitamin B12 with intrinsic factor.

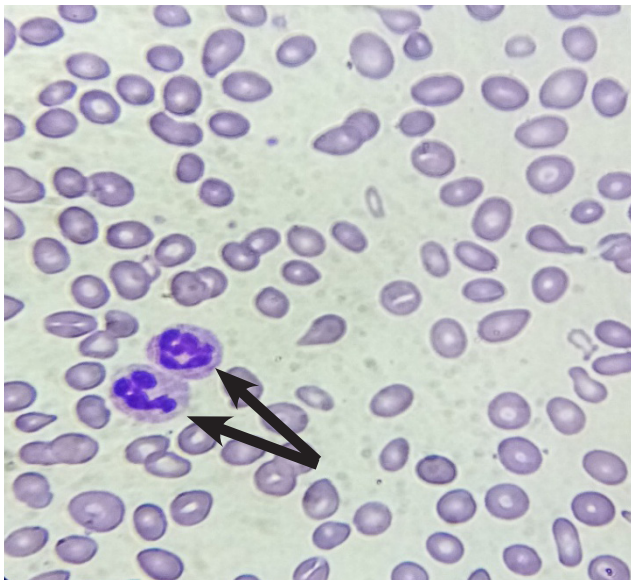


Figure (3-3) show hypersigmented neutrophils.

Red cell membrane disorders producing codocytes (nonmegaoblastic anaemia)

Other disorders which cause macrocytosis without DNA replication problems (i.e., non-megaloblastic macrocytic anemias), are disorders associated with increased red cell membrane surface area, such as pathologies of the liver and spleen which produce codocytes or "target cells" which have a central collection of hemoglobin surrounded by a pallor (a thin area) then followed by a thicker collection of hemoglobin at the rim of the cell.

Alcohol Round macrocytes which are not codocytes are produced in chronic alcoholism (which produces a mild macrocytosis even in the absence of vitamin deficiency), apparently as a direct toxic effect of alcohol specifically on the bone marrow.

Association with rapid red cell turnover and reticulocytosis

Mild macrocytosis is a common finding associated with rapid blood restoration or production, since in general, "fresh" or newly produced red cells (reticulocytes) are larger than the mean (average) size, due to slow shrinkage of normal cells over a normal red cell circulating lifetime. Thus, chronic obstructive pulmonary disease (COPD), in which red cells are rapidly produced in response to low oxygen levels in the blood, often produces mild macrocytosis. Also, rapid blood replacement from the marrow after a

traumatic blood loss, or rapid red blood cell turnover from rapid hemolysis (G6PD deficiency), also often produces mild macrocytosis in the associated anemia.

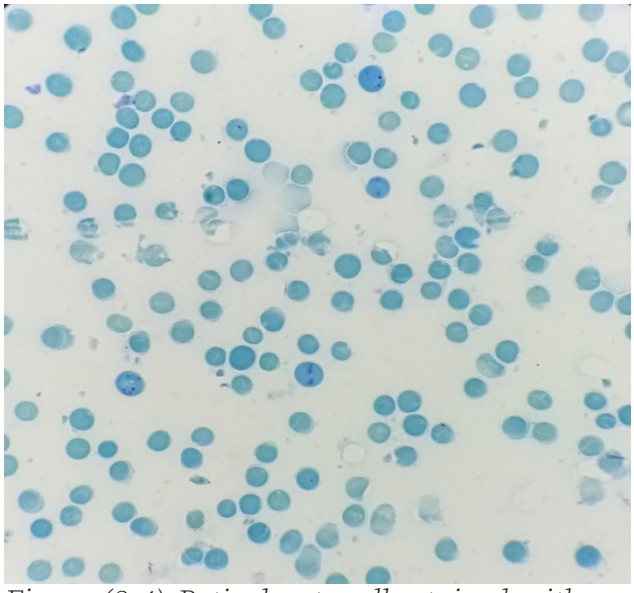


Figure (3-4) Reticulocyte cells stained with supravital stain (new methylene blue) Show blue reticulin fiber inside cells.

Anemia due to excessive Red Blood cells destruction

Hemolytic anemia

A form of anemia due to hemolysis, the abnormal breakdown of red blood cells (RBCs), either in the blood vessels (intravascular hemolysis) or elsewhere in the human body (extravascular, but usually in the spleen) (6). It has numerous possible consequences, ranging from relatively harmless to life-threatening. The general classification of hemolytic anemia is either inherited or acquired. Treatment depends on the cause and nature of the breakdown.

Symptoms of hemolytic anemia are similar to other forms of anemia (fatigue and shortness of breath), but in addition, the breakdown of red cells leads to jaundice and increases the risk of particular long-term complications, such as gallstones and pulmonary hypertension.

Signs and symptoms

In general, signs of anemia (pallor, fatigue, shortness of breath, and potential for heart failure) are present. In small children, failure to thrive may occur in any form of anemia. Certain aspects of the medical history can suggest a cause for hemolysis, such as drugs, consumption of fava beans due to Favism, the presence of prosthetic heart valve, or other medical illness.

Chronic hemolysis leads to an increased excretion of bilirubin into the biliary tract, which in turn may lead to gallstones. The continuous release of free hemoglobin has been linked with the development of pulmonary hypertension (increased pressure over the pulmonary artery); this, in turn, leads to episodes of syncope (fainting), chest pain, and progressive breathlessness. Pulmonary hypertension eventually causes right ventricular heart failure, the symptoms of which are peripheral edema (fluid accumulation in the skin of the legs) and ascites (fluid accumulation in the abdominal cavity).

Causes

They may be classified according to the means of hemolysis, being either intrinsic in cases where the cause is related to the red

blood cell (RBC) itself, or extrinsic in cases where factors external to the RBC dominate (7). Intrinsic effects may include problems with RBC proteins or oxidative stress handling, whereas external factors include immune attack and microvascular angiopathies (RBCs are mechanically damaged in circulation).

Intrinsic causes (Hereditary)

Hereditary (inherited) hemolytic anemia can be due to:

-Defects of red blood cell membrane production (as in hereditary spherocytosis, hereditary elliptocytosis and stomatocytosis)

-Defects in hemoglobin production (as in thalassemia, sickle-cell disease and congenital dyserythropoietic anemia)

-Defective red cell metabolism (as in glucose-6-phosphate dehydrogenase deficiency and pyruvate kinase deficiency)

Paroxysmal nocturnal hemoglobinuria (PNH), sometimes referred to as Marchiafava-Micheli syndrome, is a rare, acquired, potentially life-threatening disease of the blood characterized by complement-induced intravascular hemolytic anemia.

Extrinsic causes

(Acquired)

Acquired hemolytic anemia may be caused by immune-mediated causes, drugs and other miscellaneous causes.

Immune-mediated causes could include transient factors as in Mycoplasma pneumoniae infection (cold agglutinin disease) or permanent factors as in autoimmune diseases like autoimmune hemolytic anemia (itself more common in diseases such as systemic lupus erythematosus, rheumatoid arthritis, Hodgkin's lymphoma, and chronic lymphocytic leukemia).

Any of the causes of hypersplenism (increased activity of the spleen), such as portal hypertension.

Acquired hemolytic anemia is also encountered in burns and as a result of certain infections (e.g. malaria). Lead poisoning resulting from the environment causes non-immune hemolytic anemia. Similarly, poisoning by arsine or stibine also causes hemolytic anemia. Runners can suffer hemolytic anemia due to "footstrike hemolysis", owing to the destruction of red blood cells in feet at foot impact.

Low-grade hemolytic anemia occurs in 70% of prosthetic heart valve recipients, and severe hemolytic anemia occurs in 3%.

Intrinsic causes (Hereditary)

Due to Defects of red blood cell membrane production

Hereditary spherocytosis

Hereditary spherocytosis (also known

as Minkowski–Chauffard syndrome) is an abnormality of red blood cells, or erythrocytes. The disorder is caused by mutations in genes relating to membrane proteins that allow for the erythrocytes to change shape. The abnormal erythrocytes are sphere-shaped (spherocytosis) rather than the normal biconcave disk shaped. Dysfunctional membrane proteins interfere with the cell's ability to be flexible to travel from the arteries to the smaller capillaries. This difference in shape also makes the red blood cells more prone to rupture (8). Cells with these dysfunctional proteins are degraded in the spleen. This shortage of erythrocytes results in hemolytic anemia.

It was first described in 1871. It is the most common cause of inherited hemolysis in European and North American Caucasian populations, with an incidence of 1 in 5000 births. The clinical severity of HS varies from symptom-free carrier to severe hemolysis because the disorder exhibits incomplete penetrance in its expression.

Pathophysiology

Hereditary spherocytosis can be an autosomal recessive or autosomal dominant trait. Hereditary spherocytosis is most commonly (though not exclusively) found in Northern European and Japanese families, although an estimated 25% of cases are due to spontaneous mutations. A patient has a 50% chance of passing the mutation onto each of his/her offspring.

Hereditary spherocytosis is caused by a variety of molecular defects in the genes that code for the red blood cell proteins spectrin (alpha and beta), ankyrin, band 3 protein, protein 4.2, and other red blood cell membrane proteins.

These proteins are necessary to maintain the normal shape of a red blood cell, which is a biconcave disk. The integrating protein that is most commonly defective is spectrin which is responsible for incorporation and binding of spectrin, thus in its dysfunction cytoskeletal instabilities ensue.

The primary defect in hereditary spherocytosis is a deficiency of membrane surface area. Decreased surface area may be produced by two different mechanisms:

1) Defects of spectrin, ankyrin (most commonly), or protein 4.2 lead to reduced density of the membrane skeleton, destabilizing the overlying lipid bilayer and releasing band 3-containing microvesicles.

2) Defects of band 3 lead to band 3 deficiency and loss of its lipid-stabilizing effect. This results in the loss of band 3-free microvesicles.

Both pathways result in membrane loss, decreased surface area, and formation of spherocytes with decreased deformability.

As the spleen normally targets abnormally shaped red cells (which are typically older), it also destroys spherocytes. In the spleen, the passage from the cords of Billroth into the sinusoids may be seen as a bottleneck,

where red blood cells need to be flexible in order to pass through. In hereditary spherocytosis, red blood cells fail to pass through and get phagocytosed, causing extravascular hemolysis.

Symptoms

include anemia, jaundice, splenomegaly, and fatigue. Furthermore, the detritus of the broken-down blood cells – unconjugated or indirect bilirubin – accumulates in the gallbladder, and can cause pigmented gallstones to develop. In chronic patients, an infection or other illness can cause an increase in the destruction of red blood cells, resulting in the appearance of acute symptoms, a hemolytic crisis.

Acute cases can threaten to cause hypoxia through anemia and acute kernicterus through high blood levels of bilirubin, particularly in newborns. Most cases can be detected soon after birth. An adult with this disease should have their children tested, although the presence of the disease in children is usually noticed soon after birth. Occasionally, the disease will go unnoticed until the child is about 4 or 5 years of age. A person may also be a carrier of the disease and show no signs or symptoms of the disease. Other symptoms may include abdominal pain that could lead to the removal of the spleen and/or gallbladder.

Spherocytosis patients who are heterozygous for a hemochromatosis gene may suffer from iron overload, despite the hemochromatosis genes being recessive.

Complications

-Hemolytic crisis, with more pronounced jaundice due to accelerated hemolysis (may be precipitated by infection).

-Aplastic crisis with dramatic fall in hemoglobin level and (reticulocyte count)-decompensation, usually due to maturation arrest and often associated with megaloblastic changes; may be precipitated by infection, such as influenza, notably with parvovirus B19.

-Folate deficiency caused by increased bone marrow requirement.

-Pigmented gallstones occur in approximately half of untreated patients. Increased hemolysis of red blood cells leads to increased bilirubin levels, because bilirubin is a breakdown product of heme. The high levels of bilirubin must be excreted into the bile by the liver, which may cause the formation of a pigmented gallstone, which is composed of calcium bilirubinate. Since these stones contain high levels of calcium carbonates and phosphate, they are radiopaque and are visible on x-ray.

-Leg ulcer.

-Abnormally low hemoglobin A1C levels. Hemoglobin A1C (glycated hemoglobin) is a test for determining the average blood glucose levels over an extended period of time, and is often used to evaluate glucose control in diabetics. The hemoglobin A1C levels are

abnormally low because the life span of the red blood cells is decreased, providing less time for the non-enzymatic glycosylation of hemoglobin. Thus, even with high overall blood sugar, the A1C will be lower than expected

Diagnosis

On a blood smear, Howell-Jolly bodies may be seen within red blood cells. The red blood cells will appear abnormally small and lack the central pale area that is present in normal red blood cells. These changes are also seen in non-hereditary spherocytosis, but they are typically more pronounced in hereditary spherocytosis. The number of immature red blood cells (reticulocyte count) will be elevated. An increase in the mean corpuscular hemoglobin concentration is also consistent with hereditary spherocytosis.

In longstanding cases and in patients who have taken iron supplementation or received numerous blood transfusions, iron overload may be a significant problem. This is a potential cause of heart muscle damage and liver disease. Measuring iron stores is therefore considered part of the diagnostic approach to hereditary spherocytosis.

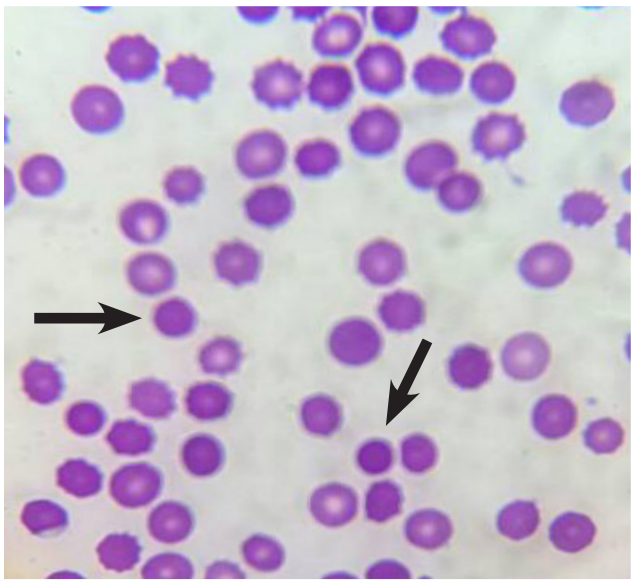


Figure (3-5) Blood film from patient with H. spherocytosis stained by Romanowsky stain shows many spherocytes.

An osmotic fragility test can aid in the diagnosis. In this test, the spherocytes will rupture in liquid solutions less concentrated than the inside of the red blood cell. This is due to increased permeability of the spherocyte membrane to salt and water, which enters the concentrated inner environment of the RBC and leads to its rupture. Although the osmotic fragility test is widely considered the gold standard for diagnosing hereditary spherocytosis, it misses as many as 25% of cases. Flow cytometric analysis of eosin-5′-maleimide-labeled intact red blood cells and the acidified glycerol lysis test are two additional options to aid diagnosis.

Treatment

Primary treatment for patients with symptomatic HS has been total splenectomy, which eliminates the hemolytic process,

allowing normal hemoglobin, reticulocyte and bilirubin levels.

Although research is ongoing, at this point there is no cure for the genetic defect that causes hereditary spherocytosis. Current management focuses on interventions that limit the severity of the disease. Treatment options include:

Splenectomy: As in non-hereditary spherocytosis, acute symptoms of anemia and hyperbilirubinemia indicate treatment with blood transfusions or exchanges and chronic symptoms of anemia and an enlarged spleen indicate dietary supplementation of folic acid and splenectomy, the surgical removal of the spleen. Splenectomy is indicated for moderate to severe cases, but not mild cases. To decrease the risk of sepsis, post-splenectomy spherocytosis patients require immunization against the influenza virus, encapsulated bacteria such as Streptococcus pneumoniae and meningococcus, and prophylactic antibiotic treatment. However, the use of prophylactic antibiotics, such as penicillin, remains controversial.

Partial splenectomy: Since the spleen is important for protecting against encapsulated organisms, sepsis caused by encapsulated organisms is a possible complication of splenectomy. The option of partial splenectomy may be considered in the interest of preserving immune function.

Surgical removal of the gallbladder may be necessary.

Hereditary elliptocytosis

Hereditary elliptocytosis, also known as ovalocytosis, is an inherited blood disorder in which an abnormally large number of the patient's erythrocytes (Red blood cells) are elliptical rather than the typical biconcave disc shape. Such morphologically distinctive erythrocytes are sometimes referred to as elliptocytes or ovalocytes. It is one of many red-cell membrane defects. In its severe forms, this disorder predisposes to haemolytic anaemia. Although pathological in humans, elliptocytosis is normal in camelids.

Pathophysiology

A number of genes have been linked to common hereditary elliptocytosis (many involve the same gene as forms of hereditary spherocytosis).These mutations have a common end result; they destabilise the cytoskeletal scaffold of cells. This stability is especially important in erythrocytes as they are constantly under the influence of deforming shear forces. As disc-shaped erythrocytes pass through capillaries, which can be 2-3 micrometres wide, they are forced to assume an elliptical shape in order to fit through. Normally, this deformation lasts only as long as a cell is present in a capillary, but in hereditary elliptocytosis the instability of the cytoskeleton means that erythrocytes deformed by passing through a capillary are forever rendered elliptical. These elliptical cells are taken up by the spleen and removed from circulation when they are younger than they would normally

be, meaning that the erythrocytes of people with hereditary elliptocytosis have a shorter than average life-span (a normal person's erythrocytes average 120 days or more).

-EL2 and EL3: The most common genetic defects (present in two-thirds of all cases of hereditary elliptocytosis) are in genes for the polypeptides α-spectrin or β-spectrin. These two polypeptides combine with one another in vivo to form an αβ heterodimer. These αβ heterodimers then combine to form spectrin tetramers. These spectrin tetramers are among the basic structural subunits of the cytoskeleton of all cells in the body. Although there is much interindividual variability, it is generally true that α-spectrin mutations result in an inability of α-spectrin to interact properly with β-spectrin to form a heterodimer. In contrast, it is generally true that β-spectrin mutations lead to αβ heterodimers being incapable of combining to form spectrin tetramers. In both cases the end result is a weakness in the cytoskeleton of the cell. Individuals with a single mutation in one of the spectrin genes are usually asymptomatic, but those who are homozygotes or are compound heterozygotes (i.e. they are heterozygous for two different elliptocytosis-causing mutations) have sufficient cell membrane instability to have a clinically significant haemolytic anaemia.

-EL1: Less common than spectrin mutations are band 4.1 mutations. Spectrin tetramers must bind to actin in order to create a proper cytoskeleton scaffold, and band 4.1 is an important protein involved in the stabilisation of the link between spectrin and actin. Similarly to the spectrin mutations, band 4.1 mutations cause a mild haemolytic anaemia in the heterozygous state, and a severe haemolytic disease in the homozygous state.

-EL4: Southeast Asian ovalocytosis is associated with the Band 3 protein.

Another group of mutations that lead to elliptocytosis are those that cause glycophorin C deficiencies. There are three phenotypes caused by abnormal glycophorin C, these are named Gerbich, Yus and Leach. Only the rarest of the three, the Leach phenotype, causes elliptocytosis. Glycophorin C has the function of holding band 4.1 to the cell membrane. It is thought that elliptocytosis in glycophorin C deficiency is actually the consequence of a band 4.1 deficit, as glycophorin C deficient individuals also have reduced intracellular band 4.1 (probably due to the reduced number of binding sites for band 4.1 in the absence of glycoprotein C).

Inheritance of multiple mutations tends to infer more serious disease. For instance, the most common genotype responsible for HPP occurs when the affected individual inherits an α-spectrin mutation from one parent (i.e. one parent has hereditary elliptocytosis) and the other parent passes on an as-yet-undefined defect that causes the affected individual's cells to preferentially produce the defective α-spectrin rather than normal α-spectrin.

Diagnosis

The diagnosis of hereditary elliptocytosis is usually made by coupling a family history of the condition with an appropriate clinical presentation and confirmation on a blood smear. In general it requires that at least 25% of erythrocytes in the specimen are abnormally elliptical in shape, though the observed percentage of elliptocytes can be 100%. This is in contrast to the rest of the population, in which it is common for up to 15% of erythrocytes to be elliptical.

If some doubt remains regarding the diagnosis, definitive diagnosis can involve osmotic fragility testing, an autohaemolysis test, and direct protein assaying by gel electrophoresis.

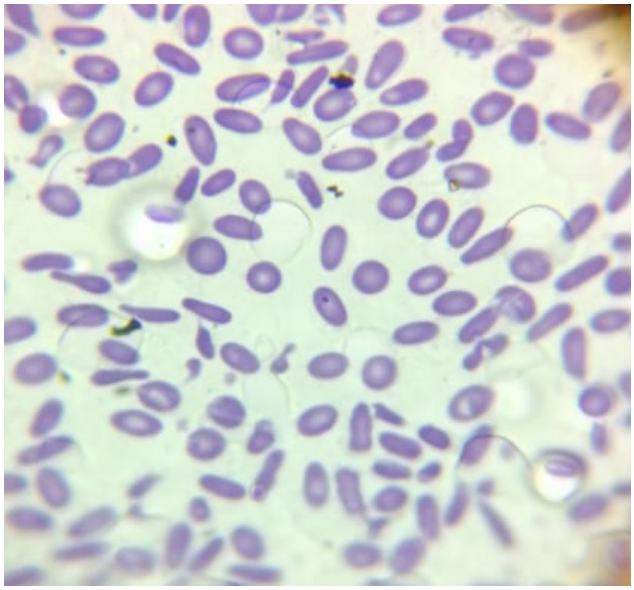


Figure (3-6) Blood film stained by Romanowsky stain shows many elliptical cells in patient with hereditary elliptocytosis.

Treatment

The vast majority of those with hereditary elliptocytosis require no treatment whatsoever. They have a mildly increased risk of developing gallstones, which is treated surgically with a cholecystectomy if pain becomes problematic. This risk is relative to the severity of the disease.

Folate helps to reduce the extent of haemolysis in those with significant haemolysis due to hereditary elliptocytosis.

Because the spleen breaks down old and worn-out blood cells, those individuals with more severe forms of hereditary elliptocytosis can have splenomegaly. Symptoms of splenomegaly can include: Vague, poorly localised abdominal pain, Fatigue and dypnea, Growth failure, Leg ulcers, Gallstones.

Removal of the spleen (splenectomy) is effective in reducing the severity of these complications, but is associated with an increased risk of overwhelming bacterial septicaemia, and is only performed on those with significant complications. Because many neonates with severe elliptocytosis progress to have only a mild disease, and because this age group is particularly susceptible to pneumococcal infections, a splenectomy is only performed on those under 5 years old when it is absolutely necessary.

Hereditary stomatocytosis

Hereditary stomatocytosis describes a number of inherited autosomal dominant human conditions which affect the red blood cell, in which the membrane or outer

coating of the cell 'leaks' sodium and potassium ions.

Causes

The cause for these hereditary conditions is now understood to be various mutations in the erythrocyte membrane protein, band 3. It is this protein which mediates the cation leaks which are characteristic of this disease.

Pathophysiology

Osmosis leads to the red blood cell having a constant tendency to swell and burst. This tendency is countered by manipulating the flow of sodium and potassium ions. A 'pump' forces sodium out of the cell and potassium in, and this action is balanced by a process called 'the passive leak'. In the hereditary stomatocytoses, the passive leak is increased and the cell becomes swamped with salt and water. The cell lyses and a haemolytic anaemia results. For as yet unknown reasons, the cells take on the shape of a cup, with a 'mouth-shaped' (stoma) area of central pallor. The two varieties of stomatocytosis classified with respect to hydration status are overhydrated (hydrocytosis) and dehydrated (xerocytosis).

Variants

Haematologists have identified a number of variants. These can be classified as below:

-Overhydrated hereditary stomatocytosis.

-Dehydrated HSt (hereditary xerocytosis; hereditary hyperphosphatidylcholine haemolytic anaemia).

-Dehydrated with perinatal ascites.

-Cryohydrocytosis.

-'Blackburn' variant.

-Familial pseudohyperkalaemia.

There are other families that do not fall neatly into any of these classifications (9).

Stomatocytosis is also found as a hereditary disease in Alaskan malamute and miniature schnauzer dogs.

Treatment

At present there is no specific treatment. Many patients with haemolytic anaemia take folic acid (vitamin B9) since the greater turnover of cells consumes this vitamin. During crises transfusion may be required. Clotting problems can occur for which anticoagulation may be needed. Unlike hereditary spherocytosis, splenectomy is contraindicated.

Hemolytic Anemia due to Defects in hemoglobin production

Thalassemia

Thalassemias are genetic disorders inherited from a person's parents, characterized by abnormal hemoglobin production. Symptoms depend on the type and can vary from

mild to severe. Anemia can result in feeling tired and pale skin. There may also be bone problems, an enlarged spleen, yellowish skin, and dark urine. Slow growth may occur in children.There are two main types, alpha thalassemia and beta thalassemia. The severity of alpha and beta thalassemia depends on how many of the four genes for alpha globin or two genes for beta globin are missing. Diagnosis is typically by blood tests including a complete blood count, special hemoglobin tests, and genetic tests. Diagnosis may occur before birth through prenatal testing.

Both α- and β-thalassemias are often inherited in an autosomal recessive manner. Cases of dominantly inherited α- and β-thalassemias have been reported, the first of which was in an Irish family with two deletions of 4 and 11 bp in exon 3 interrupted by an insertion of 5 bp in the β-globin gene. For the autosomal recessive forms of the disease, both parents must be carriers for a child to be affected. If both parents carry a hemoglobinopathy trait, the risk is 25% for each pregnancy for an affected child.

Pathophysiology

Normally, the majority of adult hemoglobin (HbA) is composed of four protein chains, two α and two β globin chains arranged into a heterotetramer. In thalassemia, patients have defects in either the α or β globin chain, causing production of abnormal red blood cells (In sickle-cell disease, the mutation is specific to β globin).

The thalassemias are classified according to which chain of the hemoglobin molecule is affected. In α-thalassemias, production of the globin chain is affected, while in β-thalassemia, production of the β globin chain is affected.

The β globin chains are encoded by a single gene on chromosome 11; α globin chains are encoded by two closely linked genes on chromosome 16. Thus, in a normal person with two copies of each chromosome, two loci encode the β chain, and four loci encode the α chain. Deletion of one of the α loci has a high prevalence in people of African or Asian descent, making them more likely to develop α-thalassemia. β-Thalassemias are not only common in Africans, but also in Greeks and Italians.

Alpha-thalassemia

The α-thalassemias involve the genes HBA1 and HBA2, inherited in a Mendelian recessive fashion. Two gene loci and so four alleles exist. It is also connected to the deletion of the 16p chromosome. α Thalassemias result in decreased alpha-globin production, therefore fewer alpha-globin chains are produced, resulting in an excess of β chains in adults and excess γ chains in newborns. The excess β chains form unstable tetramers (called hemoglobin H or HbH of 4 beta chains), which have abnormal oxygen dissociation curves.

Beta-thalassemia

Beta thalassemias are due to mutations in the HBB gene on chromosome 11, also inherited in an autosomal, recessive fashion. The severity of the disease depends on the nature of the mutation and on the presence of mutations in one or both alleles.

Mutated alleles are called β+ when partial function is conserved (either the protein has a reduced function, or it functions normally but is produced in reduced quantity) or βo, when no functioning protein is produced.

The situation of both alleles determines the clinical picture:

β thalassemia major (Mediterranean anemia or Cooley anemia) is caused by a βo/βo genotype. No functional β chains are produced, and thus no hemoglobin A can be assembled. This is the most severe form of β-thalassemia;

β thalassemia intermedia is caused by a β+/βo or β+/β+ genotype. In this form, some hemoglobin A is produced;

β thalassemia minor is caused by a β/βo or β/β+ genotype. Only one of the two β globin alleles contains a mutation, so β chain production is not terribly compromised and patients may be relatively asymptomatic.

Delta-thalassemia

As well as alpha and beta chains present in hemoglobin, about 3% of adult hemoglobin is made of alpha and delta chains. Just as with beta thalassemia, mutations that affect the ability of this gene to produce delta chains can occur.

Signs and symptoms

Iron overload: People with thalassemia can get an overload of iron in their bodies, either from the disease itself or from frequent blood transfusions. Too much iron can result in damage to the heart, liver, and endocrine system, which includes glands that produce hormones that regulate processes throughout the body. The damage is characterized by excessive deposits of iron. Without adequate iron chelation therapy, almost all patients with beta-thalassemia accumulate potentially fatal iron levels.

Infection: People with thalassemia have an increased risk of infection. This is especially true if the spleen has been removed.

Bone deformities: Thalassemia can make the bone marrow expand, which causes bones to widen. This can result in abnormal bone structure, especially in the face and skull. Bone marrow expansion also makes bones thin and brittle, increasing the risk of broken bones.

Enlarged spleen: The spleen aids in fighting infection and filters unwanted material, such as old or damaged blood cells. Thalassemia is often accompanied by the destruction of a large number of red blood cells and the task of removing these cells causes the spleen to enlarge. Splenomegaly can make anemia worse, and it can reduce the life of

transfused red blood cells. Severe enlargement of the spleen may necessitate its removal.

Slowed growth rates: anemia can cause a child's growth to slow. Puberty also may be delayed in children with thalassemia.

Heart problems: Diseases, such as congestive heart failure and abnormal heart rhythms, may be associated with severe thalassemia.

Diagnosis

Thalassemia can be diagnosed via a complete blood count, hemoglobin electrophoresis, and DNA testing.

Treatment

Treatment depends on the type and severity. Treatment for those with more severe disease often includes regular blood transfusions, iron chelation, and folic acid. Iron chelation may be done with deferoxamine or deferasirox .Occasionally, a bone marrow transplant may be an option. Complications may include iron overload from the transfusions with resulting heart or liver disease, infections, and osteoporosis. If the spleen becomes overly enlarged, surgical removal may be required.

Management of thalassemia

Mild thalassemia: people with thalassemia traits do not require medical or follow-up care after the initial diagnosis is made. People with β-thalassemia trait should be warned that their condition can be misdiagnosed as the more common iron deficiency anemia. They should avoid routine use of iron supplements; iron deficiency can develop, though, during pregnancy or from chronic bleeding. Counseling is indicated in all persons with genetic disorders, especially when the family is at risk of a severe form of disease that may be prevented.

People with severe thalassemia require medical treatment. A blood transfusion regimen was the first measure effective in prolonging life.

Iron overload multiple blood transfusions can result in iron overload. The iron overload related to thalassemia may be treated by chelation therapy with the medications deferoxamine, deferiprone, or deferasirox. These treatments have resulted in improving life expectancy in those with thalassemia major.

Deferoxamine is only effective via daily injections which makes its long-term use more difficult. It has the benefit of being inexpensive and decent long-term safety. Adverse effects are primary skin reactions around the injection site and hearing loss.

Deferasirox has the benefit of being an oral medication. Common side effects include: nausea, vomiting and diarrhea. It however is not effective in everyone and is probably not suitable in those with significant cardiac issues related to iron overload. The cost is also significant.

Deferiprone is a medication that is given by

mouth. Nausea, vomiting, and diarrhea are relatively common with its use.It is available in both Europe and the United States.It appears to be the most effective agent when the heart is significantly involved.

There is no evidence from randomized controlled trial to support zinc supplementation in thalassemia

Bone marrow transplantation

Bone marrow transplantation may offer the possibility of a cure in young people who have an HLA-matched donor. Success rates have been in the 80–90% range.Mortality from the procedure is about 3%.There are no randomized controlled trials which have tested the safety and efficacy of non-identical donor bone marrow transplantation in persons with β- thalassemia who are dependent on blood transfusion.

If the person does not have an HLA-matched compatible donor, another method called bone marrow transplantation (BMT) from haploidentical mother to child (mismatched donor) may be used. In a study of 31 people, the thalassemia-free survival rate 70%, rejection 23%, and mortality 7%. The best results are with very young people.

Epidemiology

As of 2013, thalassemia occurs in about 280 million people, with about 439,000 having severe disease. It is most common among people of Italian, Greek, Middle Eastern, South Asian, and African descent. Males and females have similar rates of disease. It resulted in 16,800 deaths in 2015, down from 36,000 deaths in 1990.Those who have minor degrees of thalassemia, similar to those with sickle-cell trait, have some protection against malaria, explaining why they are more common in regions of the world where malaria exists.

Combination hemoglobinopathies

Thalassemia can coexist with other hemoglobinopathies. The most common of these are:

Hemoglobin E/thalassemia: common in Cambodia, Thailand, and parts of India, it is clinically similar to β thalassemia major or thalassemia intermedia.

Hemoglobin S/thalassemia: common in African and Mediterranean populations, is clinically similar to sickle-cell anemia, with the additional feature of splenomegaly.

Hemoglobin C/thalassemia: common in Mediterranean and African populations, hemoglobin C/βo thalassemia causes a moderately severe hemolytic anemia with splenomegaly; hemoglobin C/β+ thalassemia produces a milder disease.

Hemoglobin D/thalassemia: common in the northwestern parts of India and Pakistan (Punjab region).

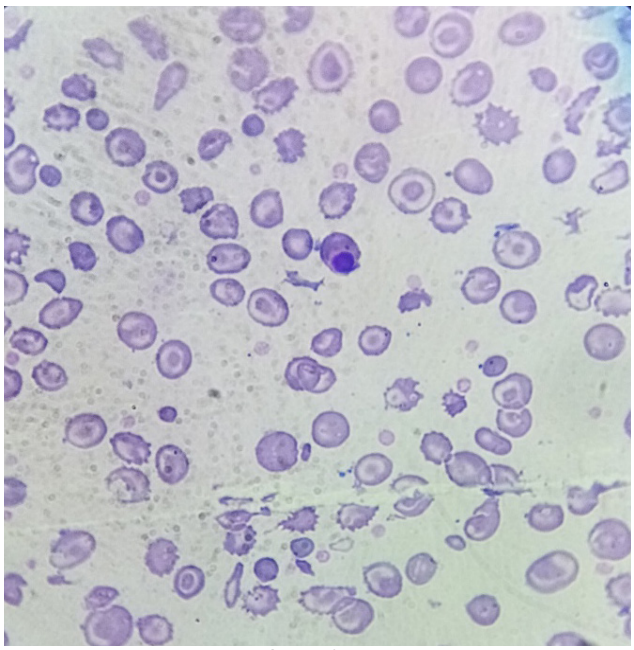


Figure (3-7) Blood film from B thalassemic patient stained by Romanowsky stain shows microcytic hypochromic cells with many target cells and fragmented RBCs and NRBCs.

Sickle cell disease

Sickle cell disease (SCD) is a group of blood disorders typically inherited from a person's parents. The most common type is known as sickle cell anaemia (SCA). It results in an abnormality in the oxygen-carrying protein haemoglobin found in red blood cells. This leads to a rigid, sickle-like shape under certain circumstances. Problems in sickle cell disease typically begin around 5 to 6 months of age. A number of health problems may develop, such as attacks of pain ("sickle cell crisis"), anemia, swelling in the hands and feet, bacterial infections and stroke. Long-term pain may develop as people get older. The average life expectancy in the developed world is 40 to 60 years.

Sickle cell disease occurs when a person inherits two abnormal copies of the haemoglobin gene, one from each parent. This gene occurs in chromosome 11. Several subtypes exist, depending on the exact mutation in each haemoglobin gene. An attack can be set off by temperature changes, stress, dehydration and high altitude. A person with a single abnormal copy does not usually have symptoms and is said to have sickle cell trait. Such people are also referred to as carriers. Diagnosis is by a blood test, and some countries test all babies at birth for the disease. Diagnosis is also possible during pregnancy.

Epidemiology

As of 2015, about 4.4 million people have sickle cell disease, while an additional 43 million have sickle cell trait. About 80% of sickle cell disease cases are believed to occur in Sub-Saharan Africa. It also occurs relatively frequently in parts of India, the Arabian Peninsula and among people of African origin living in other parts of the world. In 2015, it resulted in about 114,800 deaths. The condition was first described in the medical literature by the American physician James B. Herrick in 1910. In 1949, the genetic transmission was determined by E. A. Beet and J. V. Neel. In 1954, the protective effect against malaria of sickle cell trait was described.

Signs and symptoms

Signs of sickle cell disease usually begin in early childhood. The severity of symptoms can vary from person to person. Sickle cell disease may lead to various acute and chronic complications, several of which

have a high mortality rate.

Sickle cell crisis the terms “sickle cell crisis” or “sickling crisis” may be used to describe several independent acute conditions occurring in patients with SCD. SCD results in anaemia and crises that could be of many types including the vaso-occlusive crisis, aplastic crisis, sequestration crisis, haemolytic crisis, and others. Most episodes of sickle cell crises last between five and seven days. “Although infection, dehydration, and acidosis (all of which favor sickling) can act as triggers, in most instances, no predisposing cause is identified”.

Vaso-occlusive crisis the vaso-occlusive crisis is caused by sickle-shaped red blood cells that obstruct capillaries and restrict blood flow to an organ resulting in ischaemia, pain, necrosis, and often organ damage. The frequency, severity, and duration of these crises vary considerably. Painful crises are treated with hydration, analgesics, and blood transfusion; pain management requires opioid administration at regular intervals until the crisis has settled. For milder crises, a subgroup of patients manage on nonsteroidal anti-inflammatory drugs (NSAIDs) such as diclofenac or naproxen. For more severe crises, most patients require inpatient management for intravenous opioids; patient-controlled analgesia devices are commonly used in this setting. Vaso-occlusive crisis involving organs such as the penis or lungs are considered an emergency and treated with red-blood cell transfusions. Incentive spirometry, a technique to encourage deep breathing to minimise the development of atelectasis, is recommended.

Splenic sequestration crisis Because of its narrow vessels and function in clearing defective red blood cells, the spleen is frequently affected. It is usually infarcted before the end of childhood in individuals suffering from sickle cell anaemia. This spleen damage increases the risk of infection from encapsulated organisms; preventive antibiotics and vaccinations are recommended for those lacking proper spleen function.

Splenic sequestration crises are acute, painful enlargements of the spleen, caused by intrasplenic trapping of red cells and resulting in a precipitous fall in haemoglobin levels with the potential for hypovolemic shock. Sequestration crises are considered an emergency. If not treated, patients may die within 1–2 hours due to circulatory failure. Management is supportive, sometimes with blood transfusion. These crises are transient, they continue for 3–4 hours and may last for one day.

Acute chest syndrome acute chest syndrome (ACS) is defined by at least two of the following signs or symptoms: chest pain, fever, pulmonary infiltrate or focal abnormality, respiratory symptoms, or hypoxemia. It is the second-most common complication and it accounts for about 25% of deaths in patients with SCD, majority of cases present with vaso-occlusive crises then they develop ACS.Nevertheless, about 80% of patients

have vaso-occlusive crises during ACS.

Aplastic crisis aplastic crises are acute worsenings of the patient's baseline anaemia, producing pale appearance, fast heart rate, and fatigue. This crisis is normally triggered by parvovirus B19, which directly affects production of red blood cells by invading the red cell precursors and multiplying in and destroying them. Parvovirus infection almost completely prevents red blood cell production for two to three days. In normal individuals, this is of little consequence, but the shortened red cell life of SCD patients results in an abrupt, life-threatening situation. Reticulocyte counts drop dramatically during the disease (causing reticulocytopenia), and the rapid turnover of red cells leads to the drop in haemoglobin. This crisis takes 4 days to one week to disappear. Most patients can be managed supportively; some need blood transfusion.

Haemolytic crisis Haemolytic crises are acute accelerated drops in haemoglobin level. The red blood cells break down at a faster rate. This is particularly common in patients with coexistent G6PD deficiency. Management is supportive, sometimes with blood transfusions.

Genetics

Sickle cell disease is inherited in an autosomal recessive pattern. Normally, humans have haemoglobin A, which consists of two alpha and two beta chains, haemoglobin A2, which consists of two alpha and two delta chains, and haemoglobin F, consisting of two alpha and two gamma chains in their bodies. Out of these three types, haemoglobin F dominates until about 6 weeks of age. Afterwards, haemoglobin A dominates throughout life. In people diagnosed with sickle cell disease, at least one of the β-globin subunits in haemoglobin A is replaced with what is known as haemoglobin S. In sickle cell anaemia, a common form of sickle cell disease, haemoglobin S replaces both β-globin subunits in the haemoglobin.

Sickle cell conditions have an autosomal recessive pattern of inheritance from parents. The types of haemoglobin a person makes in the red blood cells depend on what haemoglobin genes are inherited from her or his parents. If one parent has sickle cell anaemia and the other has sickle cell trait, then the child has a 50% chance of having sickle cell disease and a 50% chance of having sickle cell trait. When both parents have sickle cell trait, a child has a 25% chance of sickle cell disease, 25% do not carry any sickle cell alleles, and 50% have the heterozygous condition.

Sickle cell gene mutation probably arose spontaneously in different geographic areas, as suggested by restriction endonuclease analysis. These variants are known as Cameroon, Senegal, Benin, Bantu, and Saudi-Asian. Their clinical importance is because some are associated with higher HbF levels, e.g., Senegal and Saudi-Asian variants, and tend to have milder disease.

The gene defect is a single nucleotide mutation (GAG codon changing to GTG) of the β-globin gene, which results in glutamic acid (E/Glu) being substituted by valine (V/Val) at position 6.Haemoglobin S with this mutation is referred to as HbS, as opposed to the normal adult HbA. This is normally a benign mutation, causing no apparent effects on the secondary, tertiary, or quaternary structures of haemoglobin in conditions of normal oxygen concentration. However, under low oxygen concentration, HbS polymerizes and forms fibrous precipitates because the deoxy form of haemoglobin exposes a hydrophobic patch on the protein between the E and F helices (Phe 85, Leu 88).

In people heterozygous for HbS (carriers of sickling haemoglobin), the polymerisation problems are minor because the normal allele is able to produce half of the haemoglobin. In people homozygous for HbS, the presence of long-chain polymers of HbS distort the shape of the red blood cell from a smooth doughnut-like shape to ragged and full of spikes, making it fragile and susceptible to breaking within capillaries. Carriers have symptoms only if they are deprived of oxygen (for example, while climbing a mountain) or while severely dehydrated.

The allele responsible for sickle cell anaemia can be found on the short arm of chromosome 11, more specifically 11p15.5. A person who receives the defective gene from both father and mother develops the disease; a person who receives one defective and one healthy allele remains healthy, but can pass on the disease and is known as a carrier or heterozygote. Heterozygotes are still able to contract malaria, but their symptoms are generally less severe.

Haemoglobin S and malaria

Due to the adaptive advantage of the heterozygote, the disease is still prevalent, especially among people with recent ancestry in malaria-stricken areas, such as Africa, the Mediterranean, India, and the Middle East. Malaria was historically endemic to southern Europe, but it was declared eradicated in the mid-20th century, with the exception of rare sporadic cases.

The malaria parasite has a complex lifecycle and spends part of it in red blood cells. In a carrier, the presence of the malaria parasite causes the red blood cells with defective haemoglobin to rupture prematurely, making the Plasmodium parasite unable to reproduce. Further, the polymerization of Hb affects the ability of the parasite to digest Hb in the first place. Therefore, in areas where malaria is a problem, people's chances of survival actually increase if they carry sickle cell trait (selection for the heterozygote).

Pathophysiology

The loss of red blood cell elasticity is central to the pathophysiology of sickle cell disease. Normal red blood cells are quite elastic, which allows the cells to deform to pass through capillaries. In sickle cell disease, low oxygen tension promotes red blood cell

sickling and repeated episodes of sickling damage the cell membrane and decrease the cell's elasticity. These cells fail to return to normal shape when normal oxygen tension is restored. As a consequence, these rigid blood cells are unable to deform as they pass through narrow capillaries, leading to vessel occlusion and ischaemia.

The actual anaemia of the illness is caused by haemolysis, the destruction of the red cells, because of their shape. Although the bone marrow attempts to compensate by creating new red cells, it does not match the rate of destruction. Healthy red blood cells typically function for 90–120 days, but sickled cells only last 10–20 days.

Diagnosis

In HbS, the complete blood count reveals haemoglobin levels in the range of 6–8 g/dl with a high reticulocyte count (as the bone marrow compensates for the destruction of sickled cells by producing more red blood cells). In other forms of sickle cell disease, Hb levels tend to be higher. A blood film may show features of hyposplenism (target cells and Howell-Jolly bodies).

Sickling of the red blood cells, on a blood film, can be induced by the addition of sodium metabisulfite. The presence of sickle haemoglobin can also be demonstrated with the "sickle solubility test". A mixture of haemoglobin S (Hb S) in a reducing solution (such as sodium dithionite) gives a turbid appearance, whereas normal Hb gives a clear solution.

Abnormal haemoglobin forms can be detected on haemoglobin electrophoresis, a form of gel electrophoresis on which the various types of haemoglobin move at varying speeds. Sickle cell haemoglobin (HgbS) and haemoglobin C with sickling (HgbSC)—the two most common forms—can be identified from there. The diagnosis can be confirmed with high-performance liquid chromatography. Genetic testing is rarely performed, as other investigations are highly specific for HbS and HbC.

An acute sickle cell crisis is often precipitated by infection. Therefore, a urinalysis to detect an occult urinary tract infection, and chest X-ray to look for occult pneumonia should be routinely performed.

People who are known carriers of the disease often undergo genetic counseling before they have a child. A test to see if an unborn child has the disease takes either a blood sample from the fetus or a sample of amniotic fluid. Since taking a blood sample from a fetus has greater risks, the latter test is usually used. Neonatal screening provides not only a method of early detection for individuals with sickle cell disease, but also allows for identification of the groups of people that carry the sickle cell trait.

Management

Treatment involves a number of measures. L-glutamine use was supported by the FDA starting at the age of 5 as it decreases com-

plications.

Folic acid and penicillin from birth to five years of age, penicillin daily, due to the immature immune system that makes them more prone to early childhood illnesses is recommended. Dietary supplementation of folic acid had been previously recommended by the WHO. A 2016 Cochrane review of its use found "the effect of supplementation on anaemia and any symptoms of anaemia remains unclear" due to a lack of medical evidence.

Malaria prevention the protective effect of sickle cell trait does not apply to people with sickle cell disease; in fact, they are more vulnerable to malaria, since the most common cause of painful crises in malarial countries is infection with malaria. It has therefore been recommended that people with sickle cell disease living in malarial countries should receive lifelong medication for prevention.

Vaso-occlusive crisis most people with sickle cell disease have intensely painful episodes called vaso-occlusive crises. However, the frequency, severity, and duration of these crises vary tremendously. Painful crises are treated symptomatically with pain medications; pain management requires opioid administration at regular intervals until the crisis has settled. For milder crises, a subgroup of patients manage on NSAIDs (such as diclofenac or naproxen). For more severe crises, most patients require inpatient management for intravenous opioids; patient-controlled analgesia (PCA) devices are commonly used in this setting. Diphenhydramine is also an effective agent that doctors frequently prescribe to help control itching associated with the use of opioids.

Acute chest crisis Management is similar to vaso-occlusive crisis, with the addition of antibiotics (usually a quinolone or macrolide, since cell wall-deficient ["atypical"] bacteria are thought to contribute to the syndrome), oxygen supplementation for hypoxia, and close observation. Should the pulmonary infiltrate worsen or the oxygen requirements increase, simple blood transfusion or exchange transfusion is indicated. The latter involves the exchange of a significant portion of the person's red cell mass for normal red cells, which decreases the percent of haemoglobin S in the patient's blood. The patient with suspected acute chest syndrome should be admitted to the hospital with worsening A-a gradient an indication for ICU admission.

Hydroxyurea The first approved drug for the causative treatment of sickle cell anaemia, hydroxyurea, was shown to decrease the number and severity of attacks to possibly increase survival time, Hydroxyurea had previously been used as a chemotherapy agent, and there is some concern that long-term use may be harmful, but this risk has been shown to be either absent or very small and it is likely that the benefits outweigh the risks.

Blood transfusions are often used in the man-

agement of sickle cell disease in acute cases and to prevent complications by decreasing the number of red blood cells (RBC) that can sickle by adding normal red blood cells. In children preventative red blood cell (RBC) transfusion therapy has been shown to reduce the risk of first stroke or silent stroke when transcranial Doppler (TCD) ultrasonography shows abnormal cerebral blood flow. In those who have sustained a prior stroke event it also reduces the risk of recurrent stroke and additional silent strokes.

Bone marrow transplants have proven effective in children. Bone marrow transplants are the only known cure for SCD. However, bone marrow transplants are difficult to obtain because of the specific HLA typing necessary. Ideally, a close relative (allogeneic) would donate the bone marrow necessary for transplantation.

Avascular necrosis when treating avascular necrosis of the bone in people with sickle cell disease, the aim of treatment is to reduce or stop the pain and maintain joint mobility. Current treatment options are to rest the joint, physical therapy, pain relief medicine, joint replacement surgery, or bone grafting. High quality randomized controlled trials are needed to assess the most effective treatment option and determine if a combination of physical therapy and surgery are more effective than physical therapy alone.

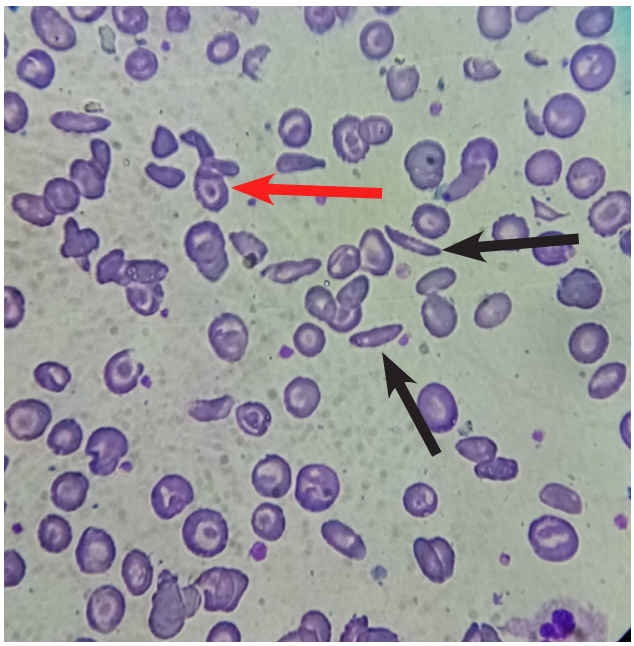


Figure (3-8) Blood film from SCD patient stained by Romanowsky stain shows normocytic normochromic cells with many sickles cells and target cells (red arrow).

Hemolytic anemia due to enzyme defect

Glucose-6-phosphate dehydrogenase deficiency

Glucose-6-phosphate dehydrogenase deficiency (G6PDD) is an inborn error of metabolism that predisposes to red blood cell breakdown. Most of the time, those who are affected have no symptoms. Following a specific trigger, symptoms such as yellowish skin, dark urine, shortness of breath, and feeling tired may develop. Complications can include anemia and newborn jaundice. Some people never have symptoms.

It is an X-linked recessive disorder that results in defective glucose-6-phosphate dehydrogenase enzyme. Red blood cell breakdown may be triggered by infections, cer-

tain medication, stress, or foods such as fava beans. Depending on the specific mutation the severity of the condition may vary.

Epidemiology

About 400 million people have the condition globally. It is particularly common in certain parts of Africa, Asia, the Mediterranean, and the Middle East. Males are affected more often than females. In 2015 it is believed to have resulted in 33,000 deaths. Carriers of the G6PDD allele may be partially protected against malaria.

Signs and symptoms

Most individuals with G6PD deficiency are asymptomatic. Symptomatic patients are almost exclusively male, due to the X-linked pattern of inheritance, but female carriers can be clinically affected due to unfavorable lyonization, where random inactivation of an X-chromosome in certain cells creates a population of G6PD-deficient red blood cells coexisting with unaffected red blood cells. A female with one affected X chromosome will show the deficiency in approximately half of her red blood cells. However, in rare cases, including double X-deficiency, the ratio can be much more than half, making the individual almost as sensitive as males.

Red blood cell breakdown (also known as hemolysis) in G6PD deficiency can manifest in a number of ways, including the following:

Prolonged neonatal jaundice, possibly leading to kernicterus (arguably the most serious complication of G6PD deficiency)

Hemolytic crises in response to: Illness (especially infections), Certain drugs , Certain foods, most notably broad beans, from which the word favism derives, Certain chemicals, Diabetic ketoacidosis.Very severe crises can cause acute kidney failure.

Favism is a hemolytic response to the consumption of fava beans, also known as broad beans. Though all individuals with favism show G6PD deficiency, not all individuals with G6PD deficiency show favism. The condition is known to be more prevalent in infants and children, and G6PD genetic variant can influence chemical sensitivity. Other than this, the specifics of the chemical relationship between favism and G6PD are not well understood.

Cause

Carriers of the underlying mutation do not show any symptoms unless their red blood cells are exposed to certain triggers, which can be:

-Foods (fava beans is the hallmark trigger for G6PD mutation carriers).

-Certain medicines including aspirin, quinine and other antimalarials derived from quinine.

-Moth balls (naphthalene).

-Stress from a bacterial or viral infection.

Drugs

Many substances are potentially harmful to people with G6PD deficiency. Variation in response to these substances makes individual predictions difficult. Antimalarial drugs that can cause acute hemolysis in people with G6PD deficiency include primaquine, pamaquine, and chloroquine. There is evidence that other antimalarials may also exacerbate G6PD deficiency, but only at higher doses. Sulfonamides (such as sulfanilamide, sulfamethoxazole, and mafenide), thiazolesulfone, methylene blue, and naphthalene should also be avoided by people with G6PD deficiency as they antagonize folate synthesis, as should certain analgesics (such as phenazopyridine and acetanilide) and a few non-sulfa antibiotics (nalidixic acid, nitrofurantoin, isoniazid, dapsone, and furazolidone). Henna has been known to cause hemolytic crisis in G6PD-deficient infants. Rasburicase is also contraindicated in G6PD deficiency. High dose intravenous vitamin C has also been known to cause haemolysis in G6PD deficiency carriers, thus G6PD deficiency testing is routine before infusion of doses of 25g or more.

Classification

The World Health Organization classifies G6PD genetic variants into five classes, the first three of which are deficiency states.

Class I: Severe deficiency (<10% activity) with chronic (nonspherocytic) hemolytic anemia.

Class II: Severe deficiency (<10% activity), with intermittent hemolysis.

Class III: Moderate deficiency (10-60% activity), hemolysis with stressors only.

Class IV: Non-deficient variant, no clinical sequelae.

Class V: Increased enzyme activity, no clinical sequelae.

6-phosphogluconate dehydrogenase (6PGD) deficiency has similar symptoms and is often mistaken for G6PD deficiency, as the affected enzyme is within the same pathway, however these diseases are not linked and can be found within the same person.

Pathophysiology

Glucose-6-phosphate dehydrogenase (G6PD) is an enzyme in the pentose phosphate pathway. G6PD converts glucose-6-phosphate into 6-phosphoglucono-δ-lactone and is the rate-limiting enzyme of this metabolic pathway that supplies reducing energy to cells by maintaining the level of the reduced form of the co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH). The NADPH in turn maintains the supply of reduced glutathione in the cells that is used to mop up free radicals that cause oxidative damage.

The G6PD / NADPH pathway is the only source of reduced glutathione in red blood cells (erythrocytes). The role of red cells as oxygen carriers puts them at substantial risk of damage from oxidizing free radicals

except for the protective effect of G6PD/NADPH/glutathione.

People with G6PD deficiency are therefore at risk of hemolytic anemia in states of oxidative stress. Oxidative stress can result from infection and from chemical exposure to medication and certain foods. Broad beans, e.g., fava beans, contain high levels of vicine, divicine, convicine and isouramil, all of which create oxidants.

When all remaining reduced glutathione is consumed, enzymes and other proteins (including hemoglobin) are subsequently damaged by the oxidants, leading to cross-bonding and protein deposition in the red cell membranes. Damaged red cells are phagocytosed and sequestered (taken out of circulation) in the spleen. The hemoglobin is metabolized to bilirubin (causing jaundice at high concentrations). The red cells rarely disintegrate in the circulation, so hemoglobin is rarely excreted directly by the kidney, but this can occur in severe cases, causing acute renal failure.

Deficiency of G6PD in the alternative pathway causes the buildup of glucose and thus there is an increase of advanced glycation endproducts (AGE). The deficiency also reduces the amount of NADPH, which is required for the formation of nitric oxide (NO). The high prevalence of diabetes mellitus type 2 and hypertension in Afro-Caribbeans in the West could be directly related to the incidence of G6PD deficiency in those populations.

Although female carriers can have a mild form of G6PD deficiency (dependent on the degree of inactivation of the unaffected X chromosome – see lyonization), homozygous females have been described; in these females there is co-incidence of a rare immune disorder termed chronic granulomatous disease (CGD).

Diagnosis

The diagnosis is generally suspected when patients from certain ethnic groups develop anemia, jaundice and symptoms of hemolysis after challenges from any of the above causes, especially when there is a positive family history.

Generally, tests will include:

Complete blood count and reticulocyte count; in active G6PD deficiency, Heinz bodies can be seen in red blood cells on a blood film;

Liver enzymes (to exclude other causes of jaundice);Lactate dehydrogenase (elevated in hemolysis and a marker of hemolytic severity)

Haptoglobin (decreased in hemolysis);

A “direct antiglobulin test” (Coombs’ test) – this should be negative, as hemolysis in G6PD is not immune-mediated;

When there are sufficient grounds to suspect G6PD, a direct test for G6PD is the “Beutler fluorescent spot test”, which has largely replaced an older test (the Motulsky dye-deco-

louration test). Other possibilities are direct DNA testing and/or sequencing of the G6PD gene.

The Beutler fluorescent spot test is a rapid and inexpensive test that visually identifies NADPH produced by G6PD under ultraviolet light. When the blood spot does not fluoresce, the test is positive; it can be falsely negative in patients who are actively hemolysing. It can therefore only be done 2–3 weeks after a hemolytic episode.

When a macrophage in the spleen identifies a RBC with a Heinz body, it removes the precipitate and a small piece of the membrane, leading to characteristic "bite cells". However, if a large number of Heinz bodies are produced, as in the case of G6PD deficiency, some Heinz bodies will nonetheless be visible when viewing RBCs that have been stained with crystal violet. This easy and inexpensive test can lead to an initial presumption of G6PD deficiency, which can be confirmed with the other tests.

Treatment

The most important measure is prevention – avoidance of the drugs and foods that cause hemolysis. Vaccination against some common pathogens (e.g. hepatitis A and hepatitis B) may prevent infection-induced attacks.

In the acute phase of hemolysis, blood transfusions might be necessary, or even dialysis in acute kidney failure. Blood transfusion is an important symptomatic measure, as the transfused red cells are generally not G6PD deficient and will live a normal lifespan in the recipient's circulation. Those affected should avoid drugs such as aspirin.

Some patients may benefit from removal of the spleen (splenectomy), as this is an important site of red cell destruction. Folic acid should be used in any disorder featuring a high red cell turnover. Although vitamin E and selenium have antioxidant properties, their use does not decrease the severity of G6PD deficiency.

Pyruvate kinase deficiency

Pyruvate kinase deficiency is an inherited metabolic disorder of the enzyme pyruvate kinase which affects the survival of red blood cells. Both autosomal dominant and recessive inheritance have been observed with the disorder; classically, and more commonly, the inheritance is autosomal recessive. Pyruvate kinase deficiency is the second most common cause of enzyme-deficient hemolytic anemia, following G6PD deficiency.

Symptoms

Symptoms can be extremely varied among those suffering from pyruvate kinase deficiency. The majority of those suffering from the disease are detected at birth while some only present symptoms during times of great physiological stress such as pregnancy, or with acute illnesses (viral disorders). Symptoms are limited to or most severe during childhood. Among the symptoms of

pyruvate kinase deficiency are: Mild to severe hemolytic Anemia- Cholecystolithiasis- Tachycardia- Hemochromatosis- Icteric sclera- Splenomegaly- Leg ulcers- Jaundice- Fatigue-Shortness of breath.

The level of 2,3-biphosphoglycerate is elevated: 1,3-biphosphoglycerate, a precursor of phosphoenolpyruvate which is the substrate for Pyruvate kinase, is increased and so the Luebering-Rapoport pathway is overactivated. This lead to a rightward shift in the oxygen dissociation curve of hemoglobin (i.e it decreases the hemoglobin affinity for oxygen): In consequence, patients may tolerate anemia surprisingly well.

Cause

Pyruvate kinase deficiency is due to a mutation in the PKLR gene. There are four pyruvate kinase isoenzymes, two of which are encoded by the PKLR gene (isoenzymes L and R, which are used in the liver and erythrocytes, respectively). Mutations in the PKLR gene therefore cause a deficiency in the pyruvate kinase enzyme.

180 different mutations have been found on the gene coding for the L and R isoenzymes, 124 of which are single-nucleotide missense mutations. Pyruvate kinase deficiency is most commonly an autosomal recessive trait. Although it is mostly homozygotes that demonstrate symptoms of the disorder, compound heterozygotes can also show clinical signs.

Pathophysiology

Pyruvate kinase is the last enzyme involved in the glycolytic process, transferring the phosphate group from phosphenol pyruvate to a waiting adenosine diphosphate (ADP) molecule, resulting in both adenosine triphosphate (ATP) and pyruvate. This is the second ATP producing step of the process and the third regulatory reaction. Pyruvate kinase deficiency in the red blood cells results in an inadequate amount of or complete lack of the enzyme, blocking the completion of the glycolytic pathway. Therefore, all products past the block would be deficient in the red blood cell. These products include ATP and pyruvate.

Mature erythrocytes lack a nucleus and mitochondria. Without a nucleus, they lack the ability to synthesize new proteins so if anything happens to their pyruvate kinase, they are unable to generate replacement enzymes throughout the rest of their life cycle. Without mitochondria, erythrocytes are heavily dependent on the anaerobic generation of ATP during glycolysis for nearly all of their energy requirements.

With insufficient ATP in an erythrocyte, all active processes in the cell come to a halt. Sodium potassium ATPase pumps are the first to stop. Since the cell membrane is more permeable to potassium than sodium, potassium leaks out. Intracellular fluid becomes hypotonic, water moves down its concentration gradient out of the cell. The cell shrinks and cellular death occurs, this is

called 'dehydration at cellular level'. This is how a deficiency in pyruvate kinase results in hemolytic anaemia, the body is deficient in red blood cells as they are destroyed by lack of ATP at a larger rate than they are being created.

Diagnosis

The diagnosis of pyruvate kinase deficiency can be done by full blood counts (differential blood counts) and reticulocyte counts. Other methods include direct enzyme assays, which can determine pyruvate kinase levels in erythrocytes separated by density centrifugation, as well as direct DNA sequencing. For the most part when dealing with pyruvate kinase deficiency, these two diagnostic techniques are complementary to each other as they both contain their own flaws. Direct enzyme assays can diagnose the disorder and molecular testing confirms the diagnosis or vice versa. Furthermore, tests to determine bile salts (bilirubin) can be used to see whether the gall bladder has been compromised.

Treatment

Most affected individuals with pyruvate kinase deficiency do not require treatment. Those individuals who are more severely affected may die in utero of anemia or may require intensive treatment. With these severe cases of pyruvate kinase deficiency in red blood cells, treatment is the only option, there is no cure. However, treatment is usually effective in reducing the severity of the symptoms.

The most common treatment is blood transfusions, especially in infants and young children. This is done if the red blood cell count has fallen to a critical level. The transplantation of bone marrow has also been conducted as a treatment option.

There is a natural way the body tries to treat this disease. It increases the erythrocyte production (reticulocytosis) because reticulocytes are immature red blood cells that still contain mitochondria and so can produce ATP via oxidative phosphorylation. Therefore, a treatment option in extremely severe cases is to perform a splenectomy. This does not stop the destruction of erythrocytes but it does help increase the amount of reticulocytes in the body since most of the hemolysis occurs when the reticulocytes are trapped in the hypoxic environment of the spleen. This reduces severe anemia and the need for blood transfusions.

Extrinsic causes (Acquired)

Acquired hemolytic anemia can be divided into immune and non-immune mediated forms of hemolytic anemia.

Immune mediated hemolytic anaemia (direct Coombs test is positive)

i. Autoimmune hemolytic anemia:

a. Warm antibody autoimmune hemolytic anemia:

-Idiopathic.

-Systemic lupus erythematosus (SLE).

-Evans' syndrome (antiplatelet antibodies and hemolytic antibodies).

b. Cold antibody autoimmune hemolytic anemia:

-Idiopathic cold hemagglutinin syndrome.

-Infectious mononucleosis and mycoplasma (atypical) pneumonia.

-Paroxysmal cold hemoglobinuria (rare).

ii. Alloimmune hemolytic anemia:

-Hemolytic disease of the newborn (HDN).

-Rh disease (Rh D).

-ABO hemolytic disease of the newborn.

-Anti-Kell hemolytic disease of the newborn.

-Rhesus c hemolytic disease of the newborn.

-Rhesus E hemolytic disease of the newborn.

-Other blood group incompatibility (RhC, Rhe, Kidd, Duffy, MN, P and others).

-Alloimmune hemolytic blood transfusion reactions (i.e., from a non-compatible blood type).

iii. Drug induced immune mediated hemolytic anemia:

Penicillin (high dose) and Methyldopa.

Non-immune mediated hemolytic anemia (direct Coombs test is negative)

1. Drugs (i.e., some drugs and other ingested substances lead to hemolysis by direct action on RBCs, e.g., ribavirin).

2. Toxins (e.g., snake venom; plant poisons such as aesculin).

3. Trauma.

4. Mechanical (from heart valves, extensive vascular surgery, microvascular disease, repeated mechanical vascular trauma).

5. Microangiopathic hemolytic anemia (a specific subtype with causes such as TTP, HUS, DIC and HELLP syndrome).

6. Infections (Note: Direct Coombs test is sometimes positive in hemolytic anemia due to infection).

7 .Septicemia.

8. Membrane disorders.

9. Paroxysmal nocturnal hemoglobinuria (rare acquired clonal disorder of red blood

cell surface proteins).

10. Liver disease.

Polycythemia

Polycythemia (also known as polycythaemia or polyglobulia) is a disease state in which the hematocrit (the volume percentage of red blood cells in the blood) is elevated.

It can be due to an increase in the number of red blood cells ("absolute polycythemia") or to a decrease in the volume of plasma ("relative polycythemia"). Polycythemia is sometimes called erythrocytosis, but the terms are not synonymous, because polycythemia refers to any increase in red blood cells, whereas erythrocytosis only refers to a documented increase of red cell mass.

Absolute polycythemia

The overproduction of red blood cells may be due to a primary process in the bone marrow (a so-called myeloproliferative syndrome), or it may be a reaction to chronically low oxygen levels or, rarely, a malignancy. Alternatively, additional red blood cells may have been received through another process—for example, being over-transfused (either accidentally or, as blood doping, deliberately) or being the recipient twin in a pregnancy, undergoing twin-to-twin transfusion syndrome.

Primary polycythemia (Polycythemia Vera)

Primary polycythemias are due to factors intrinsic to red cell precursors. Polycythemia Vera (PCV), polycythemia rubra Vera (PRV), or erythremia, occurs when excess red blood cells are produced as a result of an abnormality of the bone marrow. Often, excess white blood cells and platelets are also produced. PCV is classified as a myeloproliferative disease. Symptoms include headaches and vertigo, and signs on physical examination include an abnormally enlarged spleen and/or liver. In some cases, affected individuals may have associated conditions including high blood pressure or formation of blood clots. Transformation to acute leukemia is rare. Phlebotomy is the mainstay of treatment. A hallmark of polycythemia is an elevated hematocrit, with Hct > 55% seen in 83% of cases. A somatic (non-hereditary) mutation (V617F) in the JAK2 gene is found in 95% of cases, though also present in other myeloproliferative disorders.

Secondary polycythemia

Secondary polycythemia in which the production of erythropoietin increases appropriately is called physiologic polycythemia.

Secondary polycythemia is caused by either natural or artificial increases in the production of erythropoietin, hence an increased production of erythrocytes. In secondary polycythemia, 6 to 8 million and occasion-

ally 9 million erythrocytes may occur per millimeter of blood. Secondary polycythemia resolves when the underlying cause is treated.

Conditions which may result in a physiologically appropriate polycythemia include:

-Altitude related - This physiologic polycythemia is a normal adaptation to living at high altitudes. Many athletes train at high altitude to take advantage of this effect - a legal form of blood doping. Some individuals believe athletes with primary polycythemia may have a competitive advantage due to greater stamina. However, this has yet to be proven due to the multifaceted complications associated with this condition.

-Hypoxic disease-associated - for example in cyanotic heart disease where blood oxygen levels are reduced significantly, may also occur as a result of hypoxic lung disease .

-Iatrogenic - Secondary polycythemia can be induced directly by phlebotomy (bloodletting) to withdraw some blood, concentrate the erythrocytes, and return them to the body.

-Genetic - Heritable causes of secondary polycythemia also exist and are associated with abnormalities in hemoglobin oxygen release. This includes patients who have a special form of hemoglobin known as Hb Chesapeake, which has a greater inherent affinity for oxygen than normal adult hemoglobin. This reduces oxygen delivery to the kidneys, causing increased erythropoietin production and a resultant polycythemia. Hemoglobin Kempsey also produces a similar clinical picture. These conditions are relatively uncommon.

Conditions where the secondary polycythemia is not as a result of physiologic adaptation and occurs irrespective of body needs include:

Neoplasms - Renal cell carcinoma or liver tumors, von Hippel-Lindau disease, and endocrine abnormalities including pheochromocytoma and adrenal adenoma with Cushing's syndrome.

People whose testosterone levels are high because of the use of anabolic steroids, including athletes who abuse steroids, or people on testosterone replacement for hypogonadism or transgender hormone replacement therapy, as well as people who take erythropoietin, may develop secondary polycythemia.

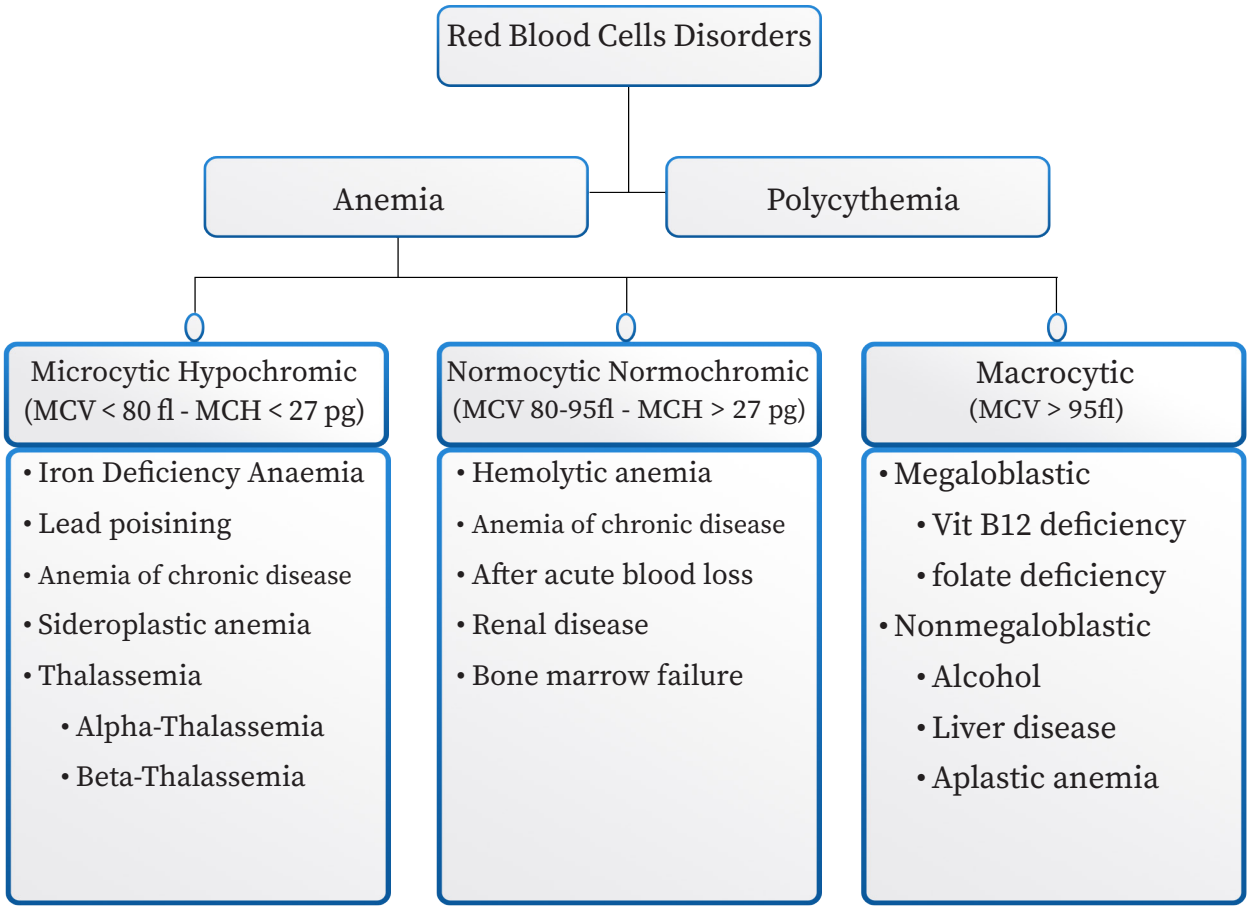
Relative polycythemia

Relative polycythemia is an apparent rise of the erythrocyte level in the blood; however, the underlying cause is reduced blood plasma (hypovolemia, cf. dehydration). Relative polycythemia is often caused by loss of body fluids, such as through burns, dehydration, and stress. A specific type of relative polycythemia is Gaisböck syndrome. In this syndrome, primarily occurring in obese men, hypertension causes a reduction in plasma volume, resulting in (amongst other changes) a relative increase in red blood cell

count

Treatment

The emergency treatment of polycythemia (e.g., in hyperviscosity or thrombosis) is by phlebotomy (removal of blood from the circulation). Depending on the underlying cause, phlebotomy may also be used on a regular basis to reduce the hematocrit. Cytostatic such as busulfan and hydroxyurea are sometimes used for long-term management of polycythemia.



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White Blood Cells Disorders

White blood cells

White blood cells help the body to fight infection. They begin life in the bone marrow and develop into different types of cells, each having a different immune purpose.

The major types are:

Neutrophils, which destroy bacteria and viruses.

Lymphocytes, which kill viruses and regulate the immune system.

Monocytes or macrophages, which eat dead or deactivated bacteria, viruses, and fungus.

Basophils and eosinophil, which help the body respond to allergic reactions and help destroy parasites.

Some white blood cell disorders impact all the different types of white blood cells in the blood, while other disorders only involve one or two specific types. Of the five types of white blood cells, neutrophils and lymphocytes get impacted the most. Most white blood cell disorders are either a type of cancer or proliferative disorders.

Proliferative disorders involve a rapid increase in the number of white blood cells that are circulating in the blood. This mostly occurs because of an infection, although, occasionally, bone marrow cancers may be responsible.

Leukopenia, however, is due to a reduction in the amount of circulating white blood cells. Leukopenia usually occurs because of:

illness

infection

toxin exposure

certain medications, such as corticosteroids or chemotherapy medications

genetic mutations

There are three major types of blood cancer that impact white blood cells, and they include the following:

Lymphoma is a type of cancer that occurs when lymphocytes change and multiply rapidly. There are two major types of lymphoma: Hodgkin's and non-Hodgkin lymphoma.

Leukemia involve the build-up of abnormal white blood cells in the bone marrow, which interferes with its ability to produce red blood cells and platelets. Leukemias can be acute and develop quickly, or chronic and develop more gradually over time.

Myelomas involve the build-up of plasma cells in the bone marrow, which interferes with the development and function of other blood cells. The most common type of myeloma is multiple myeloma, where abnormal plasma cells build-up or form a tumor in numerous locations in the bone or marrow.

Leukocytosis

Leukocytosis is an increase in white cells (the leukocyte count) more than the upper limit of the normal range in the blood according to age, sex and physiological sta-

tus. It is frequently a sign of an inflammatory response, most commonly the result of infection, but may also occur following certain parasitic infections or bone morrow tumors as well as leukemia. It may also occur after strenuous exercise, convulsions such as epilepsy, emotional stress, pregnancy and labor, anesthesia, as a side effect of medication (e.g. Lithium), and epinephrine administration.

Neutrophilia

(also called neutrophil leukocytosis or occasionally neutrocytosis) is leukocytosis of neutrophils, that is, a high number of neutrophils in the blood. Because neutrophils are the main type of granulocytes, mentions of granulocytosis often overlap in meaning with neutrophilia.

Neutrophils are the primary white blood cells that respond to a bacterial infection, so the most common cause of neutrophilia is a bacterial infection, especially pyogenic infections.

Neutrophils are also increased in any acute inflammation, so will be raised after a heart attack, other infarct or burns.

Some drugs, such as prednisone, have the same effect as cortisol and adrenaline (epinephrine), causing marginated neutrophils to enter the blood stream. Nervousness will very slightly raise the neutrophil count because of this effect.

A neutrophilia might also be the result of a malignancy. Chronic myelogenous leukemia (CML or chronic myeloid leukaemia) is a disease where the blood cells proliferate out of control. These cells may be neutrophils. Neutrophilia can be caused by appendicitis and splenectomy. Also increasing in neutrophils count can be occur physiologically during pregnancy.

Primary neutrophilia can additionally be a result of Leukocyte adhesion deficiency.

Lymphocytosis

is an increase in the number of lymphocytes in the blood. In adults, lymphocytosis is present when the lymphocyte count is greater than 4000 per microliter (4.0 x 109/L), in older children greater than 7000 per microliter and in infants greater than 9000 per microliter. Lymphocytes normally represent 20 to 40% of circulating white blood cells.

Lymphocytosis is a feature of infection, particularly in children. In the elderly, lymphoproliferative disorders, including chronic lymphocytic leukaemia and lymphomas, often present with lymphadenopathy and a lymphocytosis.

Causes of absolute lymphocytosis include

Acute viral infections, such as infectious mononucleosis (glandular fever), hepatitis and Cytomegalovirus infection.

Other acute infections such as pertussis.

Some protozoal infections, such as toxoplas-

mosis and American trypanosomiasis (Chagas disease).

Chronic intracellular bacterial infections such as tuberculosis or brucellosis.

Chronic lymphocytic leukemia.

Acute lymphoblastic leukemia.

Lymphomas.

Post-splenectomy state.

Smoking.

Causes of relative lymphocytosis include:

age less than 2 years; acute viral infections; connective tissue diseases, thyrotoxicosis, Addison's disease, and splenomegaly with splenic sequestration of granulocytes.

Lymphocytosis is usually detected when a complete blood count is obtained. If not provided the lymphocyte count can be calculated by multiplying the total white blood cell (WBC) count by the percentage of lymphocytes found in the differential count. The lymphocyte count can also be directly measured by flowcytometry.

Monocytosis is an increase in the number of monocytes circulating in the blood. Monocytes are white blood cells that give rise to macrophages and dendritic cells in the immune system. It occurs when there is a sustained rise in monocyte counts greater than 800/mm3 to 1000/mm3.

Monocytosis has sometimes been called mononucleosis, but that name is usually reserved specifically for infectious mononucleosis.

-Monocytosis often occurs during chronic inflammation. Diseases that produce such a chronic inflammatory state:

Infections: tuberculosis, brucellosis, listeriosis, subacute bacterial endocarditis, syphilis, and other viral infections and many protozoal and rickettsial infections (e.g. kala azar, malaria, Rocky Mountain spotted fever).

Blood and immune causes: chronic neutropenia and myeloproliferative disorders.

Autoimmune diseases and vasculitis: systemic lupus erythematosus, rheumatoid arthritis and inflammatory bowel disease.

Malignancies: Hodgkin's disease and certain leukaemias, such as chronic myelomonocytic leukaemia (CMML) and monocytic leukemia.

Recovery phase of neutropenia or an acute infection.

Obesity.

Miscellaneous causes: sarcoidosis and lipid storage disease.

During these stages of extreme inflammation, monocytosis can damage tissues because it increases the activation of the immune response and prevents the inflammation from subsiding which is seen in cases where sepsis occurs.

Eosinophilia

is a condition in which the eosinophil count in the peripheral blood exceeds 5.0×108/l (500/μL). Eosinophils usually account for less than 7% of the circulating leukocytes. A marked increase in non-blood tissue eosinophil count noticed upon histopathologic examination is diagnostic for tissue eosinophilia.Several causes are known, with the most common being some form of allergic reaction or parasitic infection. Diagnosis of eosinophilia is via a complete blood count (CBC), but diagnostic procedures directed at the underlying cause vary depending on the suspected condition(s). An absolute eosinophil count is not generally needed if the CBC shows marked eosinophilia. The location of the causal factor can be used to classify eosinophilia into two general types:

Extrinsic, in which the factor lies outside the eosinophil cell lineage.

Intrinsic eosinophilia, which denotes etiologies within the eosiniphil cell line.

Eosinophilia can be idiopathic (primary) or, more commonly, secondary to another disease. In the Western World, allergic or atopic diseases are the most common causes, especially those of the respiratory or integumentary systems. In the developing world, parasites are the most common cause. A parasitic infection of nearly any bodily tissue can cause eosinophilia. Diseases that feature eosinophilia as a sign include: Allergic disorders, Asthma, Hay fever, Drug allergies, Allergic skin diseases, Pemphigus, Dermatitis herpetiformis, IgG4-related disease, parasitic infections Addison's disease and stress-induced suppression of adrenal gland function. Also some forms of malignancy:

Acute lymphoblastic leukemia.

Chronic myelogenous leukemia.

Eosinophilic leukemia.

Clonal eosinophilia.

Hodgkin lymphoma.

Some forms of non-Hodgkin lymphoma.

Lymphocyte-variant hypereosinophilia.

Systemic mastocytosis.

Systemic autoimmune diseases.

Systemic lupus erythematosus.

Kimura disease.

Eosinophilic granulomatosis with polyangiitis.

Eosinophilic fasciitis.

Eosinophilic myositis.

Eosinophilic myocarditis.

Eosinophilic esophagitis.

Eosinophilic gastroenteritis.

Cholesterol embolism (transiently).

Coccidioidomycosis (Valley fever), a fungal disease prominent in the US Southwest.

Human immunodeficiency virus infection.

Interstitial nephropathy.

Hyperimmunoglobulin E syndrome, an immune disorder characterized by high levels of serum IgE.

Idiopathic hypereosinophilic syndrome.

Congenital disorders.

Hyperimmunoglobulin E syndrome.

Omenn syndrome.

Familial eosinophilia, neoplastic eosinophilia.

Drug reactions

Allergic reactions to drugs are a common cause of eosinophilia, with manifestations ranging from diffuse maculopapular rash, to severe life-threatening drug reactions with eosinophilia and systemic symptoms. Drugs that has, allopurinol, nonsteroidal anti-inflammatory drugs (NSAIDs), some antipsychotics such as risperidone, and certain antibiotics. Phenibut, an analogue of the neurotransmitter GABA, has also been implicated in high doses. The reaction which has been shown to be T-cell mediated may also cause eosinophilia-myalgia syndrome.

Diagnosis

is by complete blood count (CBC). However, in some cases, a more accurate absolute eosinophil count may be needed. Medical history is taken, with emphasis on travel, allergies and drug use. Specific test for causative conditions are performed, often including chest x-ray, urinalysis, liver and kidney function tests, and serologic tests for parasitic and connective tissue diseases. The stool is often examined for traces of parasites (i.e. eggs, larvae, etc.) though a negative test does not rule out parasitic infection; for example, trichinosis requires a muscle biopsy. Elevated serum B12 or low white blood cell alkaline phosphatase, or leukocytic abnormalities in a peripheral smear indicates a disorder of myeloproliferation. In cases of idiopathic eosinophilia, the patient is followed for complications. A brief trial of corticosteroids can be diagnostic for allergic causes, as the eosinophilia should resolve with suppression of the immune over-response.Neoplastic disorders are diagnosed through the usual methods, such as bone marrow aspiration and biopsy for the leukemias, MRI/CT to look for solid tumors, and tests for serum LDH and other tumor markers.

Leukopenia

Leukopenia (from Greek, Modern λευκός (leukos), meaning 'white', and πενία (penia), meaning 'deficiency') is a decrease in the number of leukocytes less than the lower limit of the normal range. They are the white blood cells, and are the body's primary defense against infection. Thus leukopenia places individuals at increased risk of infection.

Neutropenia, a subtype of leukopenia, refers

to a decrease in the number of circulating neutrophil granulocytes, the most abundant white blood cells. The terms leukopenia and neutropenia may occasionally be used interchangeably, as the neutrophil count is the most important indicator of infection risk. This should not be confused with agranulocytosis.

Low white cell count may be due to acute viral infections, such as a cold or influenza. It has been associated with chemotherapy, radiation therapy, myelofibrosis, aplastic anemia (failure of white cell, red cell and platelet production), stem cell transplant, bone marrow transplant, HIV, AIDS, and steroid use.

Other causes of low white blood cell count include systemic lupus erythematosus, Hodgkin's lymphoma, some types of cancer, typhoid, malaria, tuberculosis, dengue, rickettsial infections, enlargement of the spleen, folate deficiencies, psittacosis, sepsis, Sjögren's syndrome and Lyme disease. It has also been shown to be caused by deficiency in certain minerals, such as copper and zinc.

Pseudoleukopenia can develop upon the onset of infection. The leukocytes (primarily neutrophils, responding to injury first) start migrating toward the site of infection, where they can be scanned. Their migration causes bone marrow to produce more WBCs to combat infection as well as to restore the leukocytes in circulation, but as the blood sample is taken upon the onset of infection, it contains low amount of WBCs, which is why it is termed "pseudoleukopenia".

Certain medications can alter the number and function of white blood cells, Medications that can cause leukopenia include clozapine, an antipsychotic medication with a rare adverse effect leading to the total absence of all granulocytes (neutrophils, basophils, eosinophils). The antidepressant and smoking addiction treatment drug bupropion HCl (Wellbutrin) can also cause leukopenia with long-term use. Minocycline, a commonly prescribed antibiotic, is another drug known to cause leukopenia. There are also reports of leukopenia caused by divalproex sodium or valproic acid (Depakote), a drug used for epilepsy (seizures), mania (with bipolar disorder) and migraine.

The anticonvulsant drug, lamotrigine, has been associated with a decrease in white blood cell count.

The FDA monograph for metronidazole states that this medication can also cause leukopenia, and the prescriber information suggests a complete blood count, including differential cell count, before and after, in particular, high-dose therapy.

Immunosuppressive drugs, such as sirolimus, mycophenolate mofetil, tacrolimus, ciclosporin, leflunomide and TNF inhibitors, have leukopenia as a known complication. Interferons used to treat multiple sclerosis, such as interferon beta-1a and interferon beta-1b, can also cause leukopenia.

Chemotherapy targets cells that grow rapidly, such as tumors, but can also affect white blood cells, because they are characterized by bone marrow as rapid growing. A common side effect of cancer treatment is neutropenia, the lowering of neutrophils (a specific type of white blood cell).

Decreased white blood cell count may be present in cases of arsenic toxicity.

Diagnosis

Leukopenia can be identified with a complete blood count.

Leukemia

Leukemia, also spelled leukaemia, is a group of blood cancers that usually begin in the bone marrow and result in high numbers of abnormal blood cells. These blood cells are not fully developed and are called blasts or leukemia cells.

The exact cause of leukemia is unknown. A combination of genetic factors and environmental (non-inherited) factors are believed to play a role. Risk factors include smoking, ionizing radiation, some chemicals (such as benzene), prior chemotherapy, and Down syndrome. People with a family history of leukemia are also at higher risk. There are four main types of leukemia-acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL) and chronic myeloid leukemia (CML)-as well as a number of less common types. Leukemias and lymphomas both belong to a broader group of tumors that affect the blood, bone marrow, and lymphoid system, known as tumors of the hematopoietic and lymphoid tissues.

Acute lymphoblastic leukemia

Acute lymphoblastic leukemia (ALL) is a cancer of the lymphoid line of blood cells characterized by the development of large numbers of immature lymphocytes. Symptoms may include feeling tired, pale skin color, fever, easy bleeding or bruising, enlarged lymph nodes, or bone pain (1). As an acute leukemia, ALL progresses rapidly and is typically fatal within weeks or months if left untreated.

In most cases, the cause is unknown. Genetic risk factors may include Down syndrome, Li-Fraumeni syndrome, or neurofibromatosis type 1. Environmental risk factors may include significant radiation exposure or prior chemotherapy. Evidence regarding electromagnetic fields or pesticides is unclear. Some hypothesize that an abnormal immune response to a common infection may be a trigger. The underlying mechanism involves multiple genetic mutations that results in rapid cell division. The excessive immature lymphocytes in the bone marrow interfere with the production of new red blood cells, white blood cells, and platelets. Diagnosis is typically based on blood tests and bone marrow examination.

ALL is typically treated initially with chemo-

therapy aimed at bringing about remission. This is then followed by further chemotherapy typically over a number of years. Additional treatments may include intrathecal chemotherapy or radiation therapy if spread to the brain has occurred. Stem cell transplantation may be used if the disease recurs following standard treatment. Additional treatments such as immunotherapy are being studied.

ALL affected about 876,000 people globally in 2015 and resulted in about 111,000 deaths. It occurs most commonly in children, particularly those between the ages of two and five. In the United States it is the most common cause of cancer and death from cancer among children. ALL is notable for being the first disseminated cancer to be cured. Survival for children increased from under 10% in the 1960s to 90% in 2015. Survival rates remain lower for babies (50%) and adults (35%).

Signs and symptoms

Initial symptoms can be nonspecific, particularly in children. Over 50% of children with leukemia had one or more of five features: a liver one can feel (64%), a spleen one can feel (61%), pale complexion (54%), fever (53%), and bruising (52%). Additionally, recurrent infections, feeling tired, arm or leg pain, and enlarged lymph nodes can be prominent features. The B symptoms, such as fever, night sweats, and weight loss, are often present as well.

Central nervous system (CNS) symptoms such cranial neuropathies due to meningeal infiltration are identified in less than 10% of adults and less than 5% of children, particularly mature B-cell ALL (Burkitt leukemia) at presentation(2) .

The signs and symptoms of ALL are variable and include:

Generalized weakness and feeling tired

Anemia

Dizziness

Headache, vomiting, lethargy, nuchal rigidity, or cranial nerve palsies (CNS involvement)Frequent or unexplained fever and infection)

Weight loss and/or loss of appetite

Excessive and unexplained bruising

Bone pain, joint pain (caused by the spread of “blast” cells to the surface of the bone or into the joint from the marrow cavity(

Breathlessness

Enlarged lymph nodes, liver and/or spleen

Pitting edema (swelling) in the lower limbs and/or abdomen Petechiae, which are tiny red spots or lines in the skin due to low platelet levels.

Testicular enlargement

Mediastinal mass.

Causes

The cancerous cell in all is the lymphoblast. Normal lymphoblasts develop into mature, infection-fighting B-cells or T-cells, also called lymphocytes. Signals in the body control the number of lymphocytes so neither too few nor too many are made. In all, both the normal development of some lymphocytes and the control over the number of lymphoid cells become defective.

ALL emerges when a single lymphoblast gains many mutations to genes that affect blood cell development and proliferation. In childhood all, this process begins at conception with the inheritance of some of these genes. These genes, in turn, increase the risk that more mutations will occur in developing lymphoid cells. Certain genetic syndromes, like Down Syndrome, have the same effect. Environmental risk factors are also needed to help create enough genetic mutations to cause disease. Evidence for the role of the environment is seen in childhood all among twins, where only 10–15% of both genetically identical twins get all. Since they have the same genes, different environmental exposures explain why one twin gets all and the other does not.

Infant all is a rare variant that occurs in babies less than one year old. KMT2A (formerly MLL) gene rearrangements are most common and occur in the embryo or fetus before birth. These rearrangements result in increased expression of blood cell development genes by promoting gene transcription and through epigenetic changes. In contrast to childhood ALL, environmental factors are not thought to play a significant role. Aside from the KMT2A rearrangement, only one extra mutation is typically found. Environmental exposures are not needed to help create more mutations.

Risk factors

Genetic riskfactors

Common inherited risk factors include mutations in ARID5B, CDKN2A/2B, CEBPE, IKZF1, GATA3, and PIP4K2A and, more rarely, TP53. These genes play important roles in cellular development, proliferation, and differentiation. Individually, most of these mutations are low risk for all. Significant risk of disease occurs when a person inherits several of these mutations together.

The uneven distribution of genetic risk factors may help explain differences in disease rate among ethnic groups. For instance, the ARID5B mutation is less common in ethnic African populations.

Several genetic syndrome also carry increased risk of all. These include: Down syndrome, Fanconi anemia, Bloom syndrome, X-linked agammaglobulinemia, severe combined immunodeficiency, Shwachman-Diamond syndrome, Kostmann syndrome, neurofibromatosis type 1, ataxia-telangiectasia, paroxysmal nocturnal hemoglobinuria, and Li-Fraumeni syndrome. Fewer than 5% of cases are associated with a known genetic syndrome.

Rare mutations in ETV6 and PAX5 are associated with a familial form of all with autosomal dominant patterns of inheritance.

Environmental riskfactors

The environmental exposures that contribute to emergence of all is contentious and a subject of ongoing debate.

High levels of radiation exposure from nuclear fallout is a known risk factor for developing leukemia. Evidence whether less radiation, as from x-ray imaging during pregnancy, increases risk of disease remains inconclusive. Studies that have identified an association between x-ray imaging during pregnancy and all found only a slightly increased risk. Exposure to strong electromagnetic radiation from power lines has also been associated with a slightly increased risk of all. This result is questioned as no causal mechanism linking electromagnetic radiation with cancer is known.

High birth weight (greater than 4000g or 8.8lbs) is also associated with a small increased risk. The mechanism connecting high birth weight to all is also not known.

Evidence suggests that secondary leukemia can develop in individuals treated with certain types of chemotherapy, such as epipodophyllotoxins and cyclophosphamide. Delayed infection hypothesis

There is some evidence that a common infection, such as influenza, may indirectly promote emergence of all. The delayed-infection hypothesis states that all results from an abnormal immune response to infection in a person with genetic risk factors. Delayed development of the immune system due to limited disease exposure may result in excessive production of lymphocytes and increased mutation rate during an illness. Several studies have identified lower rates of all among children with greater exposure to illness early in life. Very young children who attend daycare have lower rates of all. Evidence from many other studies looking at disease exposure and all is inconclusive.

Mechanism

Several characteristic genetic changes lead to the creation of a leukemic lymphoblast. These changes include chromosomal translocations, intrachromosomal rearrangements, changes in the number of chromosomes in leukemic cells, and additional mutations in individual genes. Chromosomal translocations involve moving a large region of DNA from one chromosome to another. This move can result in placing a gene from one chromosome that promotes cell division to a more actively transcribed area on another chromosome. The result is a cell that divides more often. An example of this includes the translocation of C-MYC, a gene that encodes a transcription factor that leads to increased cell division, next to the immunoglobulin heavy- or light-chain gene enhancers, leading to increased C-MYC expression and increased cell division. Other large changes in chromosomal structure can

result in placement of two genes directly next to each other. The result is the combination of two usually separate proteins into a new fusion protein. This protein can have a new function that promotes the development of cancer. Examples of this include the ETV6-RUNX1 fusion gene that combines two factors that promote blood cell development and the BCR-ABL1 fusion gene of the Philadelphia chromosome. BCR-ABL1 encodes an always-activated tyrosine kinase that causes frequent cell division. These mutations produce a cell that divides more often, even in the absence of growth factors.

Other genetic changes in B-cell all include changes to the number of chromosomes within the leukemic cells. Gaining at least five additional chromosomes, called high hyperdiploidy, occurs more commonly. Less often, chromosomes are lost, called hypodiploidy, which is associated with a poorer prognosis. Additional common genetic changes in B-cell all involve non-inherited mutations to PAX5 and IKZF1. In T-cell ALL, LYL1, TAL1,

TLX1, and TLX3 rearrangements can occur.

ALL results when enough of these genetic changes are present in a single lymphoblast. In childhood ALL, for example, one fusion gene translocation is often found along with six to eight other ALL-related genetic changes. The initial leukemic lymphoblast copies itself into an excessive number of new lymphoblasts, none of which can develop into functioning lymphocytes. These lymphoblasts build up in the bone marrow and may spread to other sites in the body, such as lymph nodes, the mediastinum, the spleen, the testicles, and the brain, leading to the common symptoms of disease.

Diagnosis

Diagnosing ALL begins with a thorough medical history, physical examination, complete blood count, and blood smears. While many symptoms of ALL can be found in common illnesses, persistent or unexplained symptoms raise suspicion of cancer. Because many features on the medical history and exam are not specific to ALL, further testing is often needed. A large number of white blood cells and lymphoblasts in the circulating blood can be suspicious for ALL because they indicate a rapid production of lymphoid cells in the marrow. The higher these numbers typically points to a worse prognosis. While white blood cell counts at initial presentation can vary significantly, circulating lymphoblast cells are seen on peripheral blood smears in the majority of cases.

A bone marrow biopsy provides conclusive proof of ALL, typically with >20% of all cells being leukemic lymphoblasts. A lumbar puncture (also known as a spinal tap) can determine whether the spinal column and brain have been invaded. Brain and spinal column involvement can be diagnosed either through confirmation of leukemic cells in the lumbar puncture or through clinical signs of CNS leukemia as described above.

Laboratory tests that might show abnormalities include blood count, kidney function, electrolyte, and liver enzyme tests.

Pathological examination, cytogenetics (in particular the presence of Philadelphia chromosome), and immunophenotyping establish whether the leukemic cells are myeloblastic (neutrophils, eosinophils, or basophils) or lymphoblastic (B lymphocytes or T lymphocytes). Cytogenetic testing on the marrow samples can help classify disease and predict how aggressive the disease course will be. Different mutations have been associated with shorter or longer survival. Immunohistochemical testing may reveal TdT or CALLA antigens on the surface of leukemic cells. TdT is a protein expressed early in the development of pre-T and pre-B cells, whereas CALLA is an antigen found in 80% of ALL cases and also in the "blast crisis" of CML.

Medical imaging (such as ultrasound or CT scanning) can find invasion of other organs commonly the lung, liver, spleen, lymph nodes, brain, kidneys, and reproductive organs.

Immunophenotyping

In addition to cell morphology and cytogenetics, immunophenotyping, a laboratory technique used to identify proteins that are expressed on their cell surface, is a key component in the diagnosis of ALL. The preferred method of immunophenotyping is through flow cytometry. In the malignant

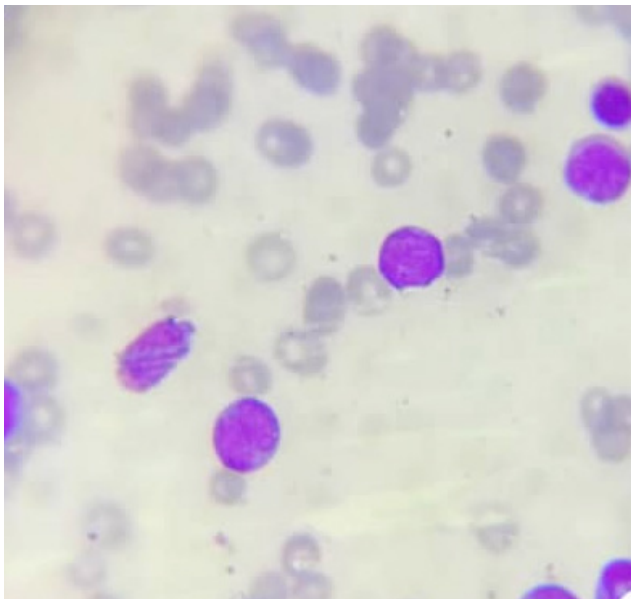

Figure (4-1) Blood film stained by Romanowsky stain shows many lymphoblast in patient with ALL.

lymphoblasts of ALL, expression of terminal deoxynucleotidyl transferase (TdT) on the cell surface can help differentiate malignant lymphocyte cells from reactive lymphocytes, white blood cells that are reacting normally to an infection in the body. On the other hand, myeloperoxidase (MPO), a marker for the myeloid lineage, is typically not expressed. Because precursor B cell and precursor T cells look the same, immunophenotyping can help differentiate the subtype of ALL and the level of maturity of the malignant white blood cells. The subtypes of ALL as determined by immunophenotype and according to the stages of maturation.

B cell Lineage

T cell Lineage

pre-pre-B ALL (pro-B-ALL)

precursor T- ALL

common ALL mature T-cell ALL

pre-B ALL

mature B-cell ALL (Burkitt leukemia - FAB L3(

An extensive panel of monoclonal antibodies to cell surface markers, particularly CD or cluster of differentiation markers, are used to classify cells by lineage. Below are immunological markers associated with B cell and T cell ALL.

Immunological Markers

B cell Lineage b. T cell Lineage

B cell Lineage

CD19, CD22, CD79a + -

CD10 - or + (common ALL(

cytoplasmic Ig - or + (pre-B ALL(

surface Ig - or + (mature B-cell ALL)

TdT + +

T cell Lineage

CD2, CD3, CD4, CD5, CD7, CD8 - +

TdT + +

Cytogenetics

Cytogenetic analysis has shown different proportions and frequencies of genetic abnormalities in cases of ALL from different age groups. This information is particularly valuable for classification and can in part explain different prognosis of these groups.

In regards to genetic analysis, cases can be stratified according to ploidy, number of sets of chromosomes in the cell, and specific genetic abnormalities, such as translocations. Hyperdiploid cells are defined as cells with more than 50 chromosomes, while hypodiploid is defined as cells with less than 44 chromosomes. Hyperdiploid cases tend to carry good prognosis while hypodiploid cases do not. For example, the most common specific abnormality in childhood B-ALL is the t (12;21) ETV6-RUNX1 translocation, in which the RUNX1 gene, encoding a protein involved in transcriptional control of hemopoiesis, has been translocated and repressed by the ETV6-RUNX1 fusion protein.

Below is a table with the frequencies of some cytogenetic translocations and molecular genetic abnormalities in ALL.

Classification

French-American-British (FAB)

Historically, prior to 2008, ALL was classified morphologically using the French-American-British (FAB) system that heavily relied on morphological assessment. The FAB system takes into account information on size, cytoplasm, nucleoli, basophilia (color of cytoplasm), and vacuolation (bubble-like properties).

While some clinicians still use the FAB scheme to describe tumor cell appearance, much of this classification has been aban-

Table (4-1): comparative between ALL subtypes according to FAB system.

FAB Subtype	Cell Type	Characteristics	Comments
ALL - L1	T cell or pre-B cell	Small and homogeneous	(uniform) cells
ALL - L2	T cell or pre-B cell	Large and heterogeneous	(varied) cells
ALL – L3	B cell	Large and varied cells with vacuoles	Mature B-cell ALL also named Burkitt leukemia. Typically, poor prognosis with standard therapy

doned because of limited impact on treatment choice and prognostic value.

World Health Organization

In 2008, the World Health Organization classification of acute lymphoblastic leukemia was developed in an attempt to create a classification system that was more clinically relevant and could produce meaningful prognostic and treatment decisions. This system recognized differences in genetic, immunophenotype, molecular, and morphological features found through cytogenetic and molecular diagnostics tests. This subtyping helps determine the prognosis and the most appropriate treatment for each specific case of ALL.

The WHO subtypes related to ALL are:

B-lymphoblastic leukemia/lymphoma

Not otherwise specified (NOS(

with recurrent genetic abnormalities

with t(9;22)(q34.1;q11.2);BCR-ABL1

with t(v;11q23.3);KMT2A rearranged

with t(12;21)(p13.2;q22.1); ETV6-RUNX1

with t(5;14)(q31.1;q32.3) IL3-IGH

with t(1;19)(q23;p13.3);TCF3-PBX1

with hyperdiploidy

with hypodiploidy

T-lymphoblastic leukemia/lymphoma

Acute leukemias of ambiguous lineage

Acute undifferentiated leukemia

Mixed phenotype acute leukemia (MPAL) with t(9;22)(q34.1;q11.2); BCR-ABL1

MPAL with t(v;11q23.3); KMT2A rearranged

MPAL, B/myeloid, NOS

MPAL, T/myeloid, NOS.

Prognosis

Prior to the development of chemotherapy regimens and hematopoietic stem cell transplant, children were surviving a median length of 3 months, largely due to either infection or bleeding. Since the advent of chemotherapy, prognosis for childhood leukemia has improved greatly and children with ALL are estimated to have a 95% probability of achieving a successful remission after 4 weeks of initiating treatment. Pediatric patients with ALL in developed countries have a greater than 80% five-year-survival rate. It is estimated that 60–80% of adults undergoing induction chemotherapy achieve complete remission after 4 weeks, and those over the age of 70 have a cure rate of 5%.

However, there are differing prognoses for ALL among individuals depending on a variety of factors:

Gender: Females tend to fare better than males.

Ethnicity: Caucasians are more likely to develop acute leukemia than African-Americans, Asians, or Hispanics. However, they also tend to have a better prognosis than non-Caucasians.

Age at diagnosis: children 1–10 years of age are most likely to develop ALL and to be cured of it. Cases in older patients are more likely to result from chromosomal abnormalities (e.g., the Philadelphia chromosome) that make treatment more difficult and prognoses poorer. Older patients are also likely to have co-morbid medical conditions that make it even more difficult to tolerate ALL treatment.

White blood cell count at diagnosis of greater than 30,000 (B-ALL) or 100,000 (T-ALL) is associated with worse outcomes

Cancer spreading into the Central nervous system (brain or spinal cord) has worse outcomes.

Morphological, immunological, and genetic subtypes

Patient's response to initial treatment and longer length of time required (greater than 4 weeks) to reach complete remission

Early relapse of ALL

Minimal residual disease

Genetic disorders, such as Down syndrome, and other chromosomal abnormalities (aneuoploidy and translocations).

Cytogenetics, the study of characteristic large changes in the chromosomes of cancer cells, is an important predictor of outcome.

Some cytogenetic subtypes have a worse prognosis than others. These include:

Patients with t(9,22) positive-ALL (30% of adult ALL cases) and other Bcr-abl-rearranged leukemias are more likely to have a poor prognosis, but survival rates may rise with treatment consisting of chemotherapy and Bcr-abl tyrosine kinase inhibitors.

A translocation between chromosomes 4 and 11 occurs in about 4% of cases and is most common in infants under 12 months.

Hyperdiploidy (>50 chromosomes) and t(12;21) are good prognostic factors and also make up 50% of pediatric ALL cases.

Unclassified ALL is considered to have an intermediate prognosis risk, somewhere in-between the good and poor risk categories.

Epidemiology

Acute lymphoblastic leukemia affected about 876,000 people and resulted in 111,000 deaths globally in 2015. It occurs in both children and adults with highest rates seen between the ages three and seven years. Around 75% of cases occur before the age of 6 with a secondary rise after the age of 40. It is estimated to affect 1 in 1500 children.

Accounting for the broad age profiles of those affected, ALL newly occurs in about 1.7 per 100,000 people per year. ALL represents approximately 20% of adult and 80% of childhood leukemias, making it the most common childhood cancer. Although 80 to 90% of children will have a long term complete response with treatment, 1527 it remains the leading cause of cancer-related deaths among children. 85% of cases are of B-cell lineage and have equal incidences in both males and females. The remaining 15% of T-cell lineage have a male predominance.

Globally ALL, typically occurs more often in Caucasians, Hispanics, and Latin Americans than in Africans. In the US, ALL is more common in children from Caucasian (36 cases/million) and Hispanic (41 cases/million) descent when compared to those from African (15 cases/million).

Acute myeloid leukemia

Acute myeloid leukemia (AML) is a cancer of the myeloid line of blood cells, characterized by the rapid growth of abnormal cells that build up in the bone marrow and blood and interfere with normal blood cells. Symptoms may include feeling tired, shortness of breath, easy bruising and bleeding, and increased risk of infection(3). Occasionally, spread may occur to the brain, skin, or gums. As an acute leukemia, AML progresses rapidly and is typically fatal within weeks or months if left untreated.

Risk factors include smoking, previous chemotherapy or radiation therapy, myelodysplastic syndrome, and exposure to the chemical benzene. The underlying mechanism involves replacement of normal bone marrow with leukemia cells, which results in a drop in red blood cells, platelets, and

normal white blood cells. Diagnosis is generally based on bone marrow aspiration and specific blood tests. AML has several subtypes for which treatments and outcomes may vary.

AML typically is initially treated with chemotherapy, with the aim of inducing remission. People may then go on to receive additional chemotherapy, radiation therapy, or a stem cell transplant. The specific genetic mutations present within the cancer cells may guide therapy, as well as determine how long that person is likely to survive. Arsenic trioxide may be tried in cases that have recurred following usual treatments.

In 2015, AML affected about one million people and resulted in 147,000 deaths globally. It most commonly occurs in older adults. Males are affected more often than females. AML is curable in about 35% of people under 60 years old and 10% over 60 years old. Older people whose health is too poor for intensive chemotherapy have a typical survival of 5–10 months. It accounts for roughly 1.8% of cancer deaths in the United States.

Singes and symptoms

Most signs and symptoms of AML are caused by the replacement of normal blood cells with leukemic cells. A lack of normal white blood cell production makes people more susceptible to infections; while the leukemic cells themselves are derived from white blood cell precursors, they have no infection-fighting capacity(4). A drop in red blood cell count (anemia) can cause fatigue, paleness, and shortness of breath. A lack of platelets can lead to easy bruising or bleeding with minor trauma.

The early signs of AML are often vague and nonspecific, and may be similar to those of influenza or other common illnesses. Some generalized symptoms include fever, fatigue, weight loss or loss of appetite, shortness of breath, anemia, easy bruising or bleeding, petechiae (flat, pin-head sized spots under the skin caused by bleeding), bone and joint pain, and persistent or frequent infections.

Enlargement of the spleen may occur in AML, but it is typically mild and asymptomatic. Lymph node swelling is rare in AML, in contrast to acute lymphoblastic leukemia. The skin is involved about 10% of the time in the form of leukemia cutis. Rarely, Sweet's syndrome, a paraneoplastic inflammation of the skin, can occur with AML.

Some people with AML may experience swelling of the gums because of infiltration of leukemic cells into the gum tissue. Rarely, the first sign of leukemia may be the development of a solid leukemic mass or tumor outside of the bone marrow, called a chloroma. Occasionally, a person may show no symptoms, and the leukemia may be discovered incidentally during a routine blood test.

Risk factors

A number of risk factors for developing AML have been identified, including: other blood disorders, chemical exposures, ionizing ra-

diation, and genetics.

Other blood disorders:

"Preleukemic" blood disorders, such as myelodysplastic syndrome (MDS) or myeloproliferative neoplasms (MPN), can evolve into AML; the exact risk depends on the type of MDS/MPN. The presence of asymptomatic clonal hematopoiesis also raises the risk of transformation into AML to 0.5–1.0% per year (5).

Chemical exposure

Exposure to anticancer chemotherapy, in particular alkylating agents, can increase the risk of subsequently developing AML. The risk is highest about three to five years after chemotherapy. Other chemotherapy agents, specifically epipodophyllotoxins and anthracyclines, have also been associated with treatment-related leukemias, which are often associated with specific chromosomal abnormalities in the leukemic cells.

Occupational chemical exposure to benzene and other aromatic organic solvents is controversial as a cause of AML. Benzene and many of its derivatives are known to be carcinogenic in vitro. While some studies have suggested a link between occupational exposure to benzene and increased risk of AML, others have suggested the attributable risk, if any, is slight.

Radiation

High amounts of ionizing radiation exposure can increase the risk of AML. Survivors of the atomic bombings of Hiroshima and Nagasaki had an increased rate of AML, as did radiologists exposed to high levels of X-rays prior to the adoption of modern radiation safety practices. People treated with ionizing radiation after treatment for prostate cancer, non-Hodgkin lymphoma, lung cancer, and breast cancer have the highest chance of acquiring AML, but this increased risk returns to the background risk observed in the general population after 12 years.

Genetics

A hereditary risk for AML appears to exist. Multiple cases of AML developing in a family at a rate higher than predicted by chance alone have been reported. Several congenital conditions may increase the risk of leukemia; the most common is probably Down syndrome, which is associated with a 10- to 18-fold increase in the risk of AML. In a second example, inactivating mutations in one of the two parental GATA2 genes lead to a reduction, i.e. a haploinsufficiency, in the cellular levels of the gene's product, the GATA2 transcription factor, and thereby to a rare autosomal dominant genetic disease, GATA2 deficiency. This disease is associated with a highly variable set of disorders including an exceedingly high risk of developing AML. The specific genetic abnormalities causing AML usually vary between those who develop the disease as a child versus an adult. However, GATA2 deficiency-induced AML may first appear in children or adults.

Diagnosis

The first clue to a diagnosis of AML is typically an abnormal result on a complete blood count. While an excess of abnormal white blood cells (leukocytosis) is a common finding with the leukemia, and leukemic blasts are sometimes seen, AML can also present with isolated decreases in platelets, red blood cells, or even with a low white blood cell count (leukopenia). While a presumptive diagnosis of AML can be made by examination of the peripheral blood smear when there are circulating leukemic blasts, a definitive diagnosis usually requires an adequate bone marrow aspiration and biopsy as well as ruling out pernicious anemia (Vitamin B12 deficiency), folic acid deficiency and copper deficiency.

Marrow or blood is examined under light microscopy, as well as flow cytometry, to diagnose the presence of leukemia, to differentiate AML from other types of leukemia (e.g. acute lymphoblastic leukemia – ALL), and to classify the subtype of disease. A sample of marrow or blood is typically also tested for chromosomal abnormalities by routine cytogenetics or fluorescent in situ hybridization. Genetic studies may also be performed to look for specific mutations in genes such as FLT3, nucleophosmin, and KIT, which may influence the outcome of the disease.

Cytochemical stains on blood and bone marrow smears are helpful in the distinction of AML from ALL, and in subclassification of AML. The combination of a myeloperoxidase or Sudan black stain and a nonspecific esterase stain will provide the desired information in most cases. The myeloperoxidase or Sudan black reactions are most useful in establishing the identity of AML and distinguishing it from ALL. The nonspecific esterase stain is used to identify a monocytic component in AMLs and to distinguish a poorly differentiated monoblastic leukemia from ALL.

The diagnosis and classification of AML can be challenging, and should be performed by a qualified hematopathologist or hematologist. In straightforward cases, the presence of certain morphologic features (such as Auer rods) or specific flow cytometry results can distinguish AML from other leukemias; however, in the absence of such features, diagnosis may be more difficult.

The two most commonly used classification schemata for AML are the older French-American-British (FAB) system and the newer World Health Organization (WHO) system. According to the widely used WHO criteria, the diagnosis of AML is established by demonstrating involvement of more than 20% of the blood and/or bone marrow by leukemic myeloblasts, except in the three best prognosis forms of acute myeloid leukemia with recurrent genetic abnormalities (t(8;21), inv(16), and t(15;17)) in which the presence of the genetic abnormality is diagnostic irrespective of blast percent. The French–American–British (FAB) classification is a bit more stringent, requiring a blast

percentage of at least 30% in bone marrow (BM) or peripheral blood (PB) for the diagnosis of AML. AML must be carefully differentiated from "preleukemic" conditions such as myelodysplastic or myeloproliferative syndromes, which are treated differently.

Because acute promyelocytic leukemia (APL) has the highest curability and requires a unique form of treatment, it is important to quickly establish or exclude the diagnosis of this subtype of leukemia. Fluorescent in situ hybridization performed on blood or bone marrow is often used for this purpose, as it readily identifies the chromosomal translocation [t(15;17)(q22;q12);] that characterizes APL. There is also a need to molecularly detect the presence of PML/RARA fusion protein, which is an oncogenic product of that translocation.

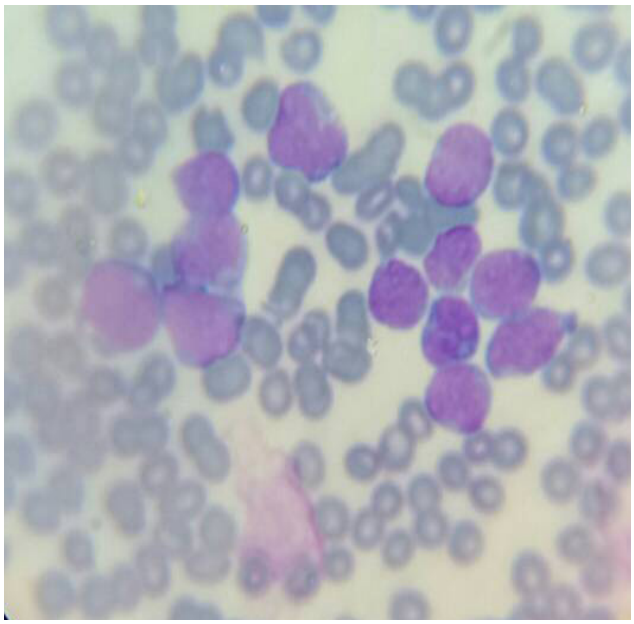

Figure (4-2) Blood film stained by Romanowsky stain shows many myeloblast in patient with AML.

The French-American-British (FAB) classification of AML

In the 1970s, a group of French, American, and British leukemia experts divided AML into subtypes, M0 through M7, based on the type of cell the leukemia develops from and how mature the cells are. This was based largely on how the leukemia cells looked under the microscope after routine staining.

M0; Undifferentiated acute myeloblastic leukemia

M1; Acute myeloblastic leukemia with minimal maturation

M2; Acute myeloblastic leukemia with maturation

M3; Acute promyelocytic leukemia (APL)

M4; Acute myelomonocytic leukemia

M4 eos; Acute myelomonocytic leukemia with eosinophilia

M5; Acute monocytic leukemia

M6; Acute erythroid leukemia

M7; Acute megakaryoblastic leukemia

Subtypes M0 through M5 all start in immature forms of white blood cells. M6 AML starts in very immature forms of red blood cells, while M7 AML starts in immature forms of cells that make platelets.

Epidemiology

Acute myeloid leukemia is a relatively rare cancer. There are approximately 10,500 new

cases each year in the United States, and the incidence rate has remained stable from 1995 through 2005. AML accounts for 1.2% of all cancer deaths in the United States.

The incidence of AML increases with age; the median age at diagnosis is 63 years. AML accounts for about 90% of all acute leukemias in adults, but is rare in children. The rate of therapy-related AML (that is, AML caused by previous chemotherapy) is rising; therapy-related disease currently accounts for about 10–20% of all cases of AML. AML is slightly more common in men, with a male-to-female ratio of 1.3:1.

There is some geographic variation in the incidence of AML. In adults, the highest rates are seen in North America, Europe, and Oceania, while adult AML is rarer in Asia and Latin America. In contrast, childhood AML is less common in North America and India than in other parts of Asia. These differences may be due to population genetics, environmental factors, or a combination of the two.

AML accounts for 34% of all leukemia cases in the UK, and around 2,900 people were diagnosed with the disease in 2011.

Pathophysiology

The malignant cell in AML is the myeloblast. In normal hematopoiesis, the myeloblast is an immature precursor of myeloid white blood cells; a normal myeloblast will gradually mature into a mature white blood cell. In AML, though, a single myeloblast accumulates genetic changes which "freeze" the cell in its immature state and prevent differentiation. Such a mutation alone does not cause leukemia; however, when such a "differentiation arrest" is combined with other mutations which disrupt genes controlling proliferation, the result is the uncontrolled growth of an immature clone of cells, leading to the clinical entity of AML.

Much of the diversity and heterogeneity of AML is because leukemic transformation can occur at a number of different steps along the differentiation pathway. Modern classification schemes for AML recognize that the characteristics and behavior of the leukemic cell (and the leukemia) may depend on the stage at which differentiation was halted.

Specific cytogenetic abnormalities can be found in many people with AML; the types of chromosomal abnormalities often have prognostic significance. The chromosomal translocations encode abnormal fusion proteins, usually transcription factors whose altered properties may cause the "differentiation arrest". For example, in acute promyelocytic leukemia, the t(15;17) translocation produces a PML-RARα fusion protein which binds to the retinoic acid receptor element in the promoters of several myeloid-specific genes and inhibits myeloid differentiation.

The clinical signs and symptoms of AML result from the growth of leukemic clone cells, which tends to displace or interfere with the development of normal blood cells

in the bone marrow. This leads to neutropenia, anemia, and thrombocytopenia. The symptoms of AML are, in turn, often due to the low numbers of these normal blood elements. In rare cases, people with AML can develop a chloroma, or solid tumor of leukemic cells outside the bone marrow, which can cause various symptoms depending on its location.

An important pathophysiological mechanism of leukemogenesis in AML is the epigenetic induction of dedifferentiation by genetic mutations that alter the function of epigenetic enzymes, such as the DNA demethylase TET2 and the metabolic enzymes IDH1 and IDH2, which lead to the generation of a novel oncometabolite, D-2-hydroxyglutarate, which inhibits the activity of epigenetic enzymes such as TET2. The hypothesis is that such epigenetic mutations lead to the silencing of tumor suppressor genes and/or the activation of proto-oncogenes.

Treatment

First-line treatment of AML consists primarily of chemotherapy, and is divided into two phases: induction and postremission (or consolidation) therapy. The goal of induction therapy is to achieve a complete remission by reducing the number of leukemic cells to an undetectable level; the goal of consolidation therapy is to eliminate any residual undetectable disease and achieve a cure. Hematopoietic stem cell transplantation is usually considered if induction chemotherapy fails or after a person relapses, although transplantation is also sometimes used as front-line therapy for people with high-risk disease. Efforts to use tyrosine kinase inhibitors in AML continue.

Prognosis

Acute myeloid leukemia is a curable disease; the chance of cure for a specific person depends on a number of prognostic factors.

Pregnancy

Leukemia is rarely associated with pregnancy, affecting only about 1 in 10,000 pregnant women. How it is handled depends primarily on the type of leukemia. Acute leukemias normally require prompt, aggressive treatment, despite significant risks of pregnancy loss and birth defects, especially if chemotherapy is given during the developmentally sensitive first trimester

Myeloproliferative neoplasms

The myeloproliferative neoplasms (MPNs), previously myeloproliferative diseases (MPDs), are a group of diseases of the bone marrow in which excess cells are produced. They are related to, and may evolve into, myelodysplastic syndrome and acute myeloid leukemia, although the myeloproliferative diseases on the whole have a much better prognosis than these conditions. The concept of myeloproliferative disease was first proposed in 1951 by the hematologist William Dameshek. In the most recent World Health Organization classification of hema-

tologic malignancies, this group of diseases was renamed from "myeloproliferative diseases" to "myeloproliferative neoplasms". This reflects the underlying clonal genetic changes that are a salient feature of this group of disease.

The increased numbers of blood cells may not cause any symptoms, but a number of medical problems or symptoms may occur. The risk of thrombosis is increased in some types of MPN.

Classification

Although a malignant neoplasm like other cancers, MPNs are classified within the hematological neoplasms. There are four main myeloproliferative diseases, which can be further categorized by the presence of the Philadelphia chromosome:

Philadelphia chromosome positive:

Chronic myelogenous leukemia (CML)

Polycythemia vera (PV(

Myelofibrosis (MF(

Philadelphia chromosome negative:

Essential thrombocythemia (ET(

In 2008, the World Health Organization listed these diagnoses as types of MPD:

Chronic myelogenous leukemia (BCR-ABL1–positive)

Chronic neutrophilic leukemia

Polycythemia vera

Primary myelofibrosis

Essential thrombocythemia

Chronic eosinophilic leukemia (not otherwise specified)

Mastocytosis

Causes

All MPNs arise from precursors of the myeloid lineages in the bone marrow. The lymphoid lineage may produce similar diseases, the lymphoproliferative disorders (acute lymphoblastic leukemia, lymphomas, chronic lymphocytic leukemia and multiple myeloma.

Most Philadelphia chromosome negative cases have an activating JAK2 or MPL mutation. Mutations in CALR have been found in the majority of JAK2 and MPL-negative essential thrombocythemia and myelofibrosis. In 2005, the discovery of the JAK2V617F mutation provided the first evidence that a fraction of persons with these disorders have a common molecular pathogenesis. Patients with JAK2V617F-negative polycythemia vera are instead positive for another class of activating JAK2 mutations - the JAK2 exon 12 mutations.

A subset may additionally have mutations in the genes LNK, CBL, TET2, ASXL1, IDH, IKZF1 or EZH2; the pathogenetic contribution of these mutations is being studied.

Diagnosis

Depending on the nature of the myelopro-

liferative neoplasm, diagnostic tests may include red cell mass determination (for polycythemia), bone marrow aspirate and trephine biopsy, arterial oxygen saturation and carboxyhaemoglobin level, neutrophil alkaline phosphatase level, vitamin B12 (or B12 binding capacity), serum urate or direct sequencing of the patient's DNA.

According to the WHO Classification of Hematopoietic and Lymphoid Neoplasms 2008 myeloproliferative neoplasms are divided into categories by diagnostic characteristics as follows:

Chronic myeloid leukemia

Chronic myeloid leukemia (CML), also known as chronic myelogenous leukemia, is a cancer of the white blood cells. It is a form of leukemia characterized by the increased and unregulated growth of myeloid cells in the bone marrow and the accumulation of these cells in the blood. CML is a clonal bone marrow stem cell disorder in which a proliferation of mature granulocytes (neutrophils, eosinophils and basophils) and their precursors is found. It is a type of myeloproliferative neoplasm associated with a characteristic chromosomal translocation called the Philadelphia chromosome.

CML is largely treated with targeted drugs called tyrosine-kinase inhibitors (TKIs) which have led to dramatically improved long-term survival rates since 2001. These drugs have revolutionized treatment of this disease and allow most patients to have a good quality of life when compared to the former chemotherapy drugs. In Western countries, CML accounts for 15–25% of all adult leukemias and 14% of leukemias overall (including the pediatric population, where CML is less common) (6).

Signs and symptoms

The way CML presents depends on the stage of the disease at diagnosis as it has been known to skip stages in some cases.

Most patients (~90%) are diagnosed during the chronic stage which is most often asymptomatic. In these cases it may be diagnosed incidentally with an elevated white blood cell count on a routine laboratory test. It can also present with symptoms indicative of hepatosplenomegaly and the resulting upper quadrant pain this causes. The enlarged spleen may put pressure on the stomach causing a loss of appetite and resulting weight loss. It may also present with mild fever and night sweats due to an elevated basal level of metabolism.

Some (<10%) are diagnosed during the accelerated stage which most often presents bleeding, petechiae and ecchymosis. In these patients fevers are most commonly the result of opportunistic infections.

Some patients are initially diagnosed in the blast phase in which the symptoms are most likely fever, bone pain and an increase in bone marrow fibrosis.

Cause

In most cases no obvious cause for CML can be isolated.

Riskfactors

CML is more common in males than in females (male to female ratio of 1.4:1) and appears more commonly in the elderly with a median age at diagnosis of 65 years. Exposure to ionising radiation appears to be a risk factor, based on a 50 fold higher incidence of CML in Hiroshima and Nagasaki nuclear bombing survivors. The rate of CML in these individuals seems to peak about 10 years after the exposure.

Classification

CML is often divided into three phases based on clinical characteristics and laboratory findings. In the absence of intervention, CML typically begins in the chronic phase, and over the course of several years progresses to an accelerated phase and ultimately to a blast crisis. Blast crisis is the terminal phase of CML and clinically behaves like an acute leukemia. Drug treatment will usually stop this progression if started early. One of the drivers of the progression from chronic phase through acceleration and blast crisis is the acquisition of new chromosomal abnormalities (in addition to the Philadelphia chromosome). Some patients may already be in the accelerated phase or blast crisis by the time they are diagnosed.

Pathophysiology

CML was the first cancer to be linked to a clear genetic abnormality, the chromosomal translocation known as the Philadelphia chromosome. This chromosomal abnormality is so named because it was first discovered and described in 1960 by two scientists from Philadelphia, Pennsylvania, USA: Peter Nowell of the University of Pennsylvania and David Hungerford of Fox Chase Cancer Center (7).

In this translocation, parts of two chromosomes (the 9th and 22nd) switch places. As a result, part of the BCR ("breakpoint cluster region") gene from chromosome 22 is fused with the ABL gene on chromosome 9. This abnormal "fusion" gene generates a protein of p210 or sometimes p185 weight (p210 is short for 210 kDa protein, a shorthand used for characterizing proteins based solely on size). Because abl carries a domain that can add phosphate groups to tyrosine residues (a tyrosine kinase), the bcr-abl fusion gene product is also a tyrosine kinase.

The fused BCR-ABL protein interacts with the interleukin 3beta(c) receptor subunit. The BCR-ABL transcript is continuously active and does not require activation by other cellular messaging proteins. In turn, BCR-ABL activates a cascade of proteins that control the cell cycle, speeding up cell division. Moreover, the BCR-ABL protein inhibits DNA repair, causing genomic instability and making the cell more susceptible to developing further genetic abnormalities. The action of

the BCR-ABL protein is the pathophysiologic cause of chronic myelogenous leukemia. With improved understanding of the nature of the BCR-ABL protein and its action as a tyrosine kinase, targeted therapies (the first of which was imatinib) that specifically inhibit the activity of the BCR-ABL protein have been developed. These tyrosine kinase inhibitors can induce complete remissions in CML, confirming the central importance of bcr-abl as the cause of CML.

CML is often suspected on the basis of a complete blood count, which shows increased granulocytes of all types, typically including mature myeloid cells. Basophils and eosinophils are almost universally increased; this feature may help differentiate CML from a leukemoid reaction. A bone marrow biopsy is often performed as part of the evaluation for CML, and CML is diagnosed by cytogenetics that detects the translocation t(9;22)(q34;q11.2) which involves the ABL1 gene in chromosome 9 and the BCR gene in chromosome 22. As a result of this translocation, the chromosome looks smaller than its homologue chromosome, and this appearance is known as the Philadelphia chromosome chromosomal abnormality. Thus, this abnormality can be detected by routine cytogenetics, and the involved genes BCR-ABL1 can be detected by fluorescent in situ hybridization, as well as by PCR.

Controversy exists over so-called Ph-negative CML, or cases of suspected CML in which the Philadelphia chromosome cannot be detected. Many such patients in fact have complex chromosomal abnormalities that mask the (9; 22) translocation, or have evidence of the translocation by FISH or RT-PCR in spite of normal routine karyotyping. The small subset of patients without detectable molecular evidence of BCR-ABL1 fusion may be better classified as having an undifferentiated myelodysplastic/myeloproliferative disorder, as their clinical course tends to be different from patients with CML.

CML must be distinguished from a leukemoid reaction, which can have a

similar appearance on a blood smear.

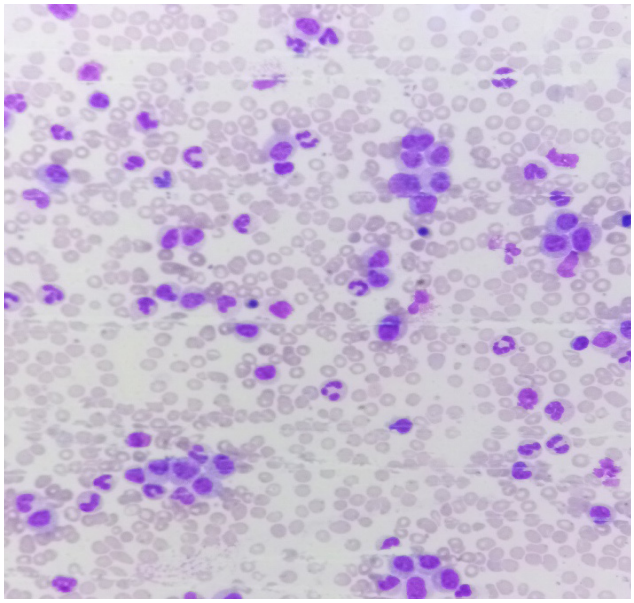

Figure (4-3) Blood film stained by Romanowsky stain shows many full myeloid spectrum in patient with CML (40x).

Chronic phase

Approximately 85% of patients with CML are in the chronic phase at the time of diagnosis. During this phase, patients are usually asymptomatic or have only mild symptoms

of fatigue, left side pain, joint and/or hip pain, or abdominal fullness. The duration of chronic phase is variable and depends on how early the disease was diagnosed as well as the therapies used. In the absence of treatment, the disease progresses to an accelerated phase. Precise patient staging based on clinical markers and personal genomic profile will likely prove beneficial in the assessment of disease history with respect to progression risk.

Accelerated phase

Criteria for diagnosing transition into the accelerated phase are somewhat variable; the most widely used criteria are those put forward by investigators at M.D. Anderson Cancer Center, by Sokal et al., and the World Health Organization. The WHO criteria are perhaps most widely used, and define the accelerated phase by the presence of ≥1 of the following haematological/cytogenetic criteria or provisional criteria concerning response to tyrosine kinase inhibitor (TKI) therapy

Haematological / cytogenetic criteria

Persistent or increasing high white blood cell count (> 10 × 109/L), unresponsive to therapy

Persistent or increasing splenomegaly, unresponsive to therapy

Persistent thrombocytosis (> 1000 × 109/L), unresponsive to therapy

Persistent thrombocytopenia (< 100 × 109/L), unrelated to therapy

≥ 20% basophils in the peripheral blood

10—19% blasts in the peripheral blood and/or bone marrow

Additional clonal chromosomal abnormalities in Philadelphia (Ph) chromosome-positive (Ph+) cells at diagnosis, including so-called major route abnormalities (a second Ph chromosome, trisomy 8, isochromosome 17q, trisomy 19), complex karyotype, and abnormalities of 3q26.2

Any new clonal chromosomal abnormality in Ph+ cells that occurs during therapy

Provisional response-to-TKI criteria

Haematological resistance (or failure to achieve a complete haematological response d) to the first TKI

Any haematological, cytogenetic, or molecular indications of resistance to two sequential TKIs

Occurrence of two or more mutations in the BCR-ABL1 fusion gene during TKI therapy

The patient is considered to be in the accelerated phase if any of the above are present. The accelerated phase is significant because it signals that the disease is progressing and transformation to blast crisis is imminent. Drug treatment often becomes less effective in the advanced stages.

Blastcrisis

Blast crisis is the final phase in the evolution

of CML, and behaves like an acute leukemia, with rapid progression and short survival. Blast crisis is diagnosed if any of the following are present in a patient with CML:

>20% blasts in the blood or bone marrow

The presence of an extramedullary proliferation of blasts

Treatment

The only curative treatment for CML is a bone marrow transplant or an allogeneic stem cell transplant. Other than this there are four major mainstays of treatment in CML: treatment with tyrosine kinase inhibitors, myelosuppressive or leukopheresis therapy (to counteract the leukocytosis during early treatment), splenectomy and interferon alfa-2b treatment. Due to the high median age of patients with CML it is relatively rare for CML to be seen in pregnant women, despite this, however, chronic myelogenous leukemia can be treated with relative safety at any time during pregnancy with Interferon-alpha hormones.

Chronic phase

In the past, antimetabolites (e.g., cytarabine, hydroxyurea), alkylating agents, interferon alfa 2b, and steroids were used as treatments of CML in the chronic phase, but since the 2000s have been replaced by BCR-ABL tyrosine-kinase inhibitors drugs that specifically target BCR-ABL, the constitutively activated tyrosine kinase fusion protein caused by the Philadelphia chromosome translocation. Despite the move to replacing cytotoxic antineoplastics (standard anticancer drugs) with tyrosine kinase inhibitors sometimes hydroxyurea is still used to counteract the high leukocyte counts encountered during treatment with tyrosine kinase inhibitors like imatinib; in these situations it may be the preferred myelosuppressive agent due to its relative lack of leukemogenic effects and hence the relative lack of potential for secondary hematologic malignancies to result from treatment. IRIS, an international study that compared interferon/cytarabine combination and the first of these new drugs imatinib, with long-term follow up, demonstrated the clear superiority of tyrosine-kinase-targeted inhibition over existing treatments.

Imatinib

The first of this new class of drugs was imatinib mesylate (marketed as Gleevec or Glivec), approved by the U.S. Food and Drug Administration (FDA) in 2001. Imatinib was found to inhibit the progression of CML in the majority of patients (65–75%) sufficiently to achieve regrowth of their normal bone marrow stem cell population (a cytogenetic response) with stable proportions of maturing white blood cells. Because some leukemic cells (as evaluated by RT-PCR) persist in nearly all patients, the treatment has to be continued indefinitely. Since the advent of imatinib, CML has become the first cancer in which a standard medical treatment may give to the patient a normal life expectancy.

Dasatinib, nilotinib, radotinib and bosutinib

To overcome imatinib resistance and to increase responsiveness to TK inhibitors, four novel agents were later developed. The first, dasatinib, blocks several further oncogenic proteins, in addition to more potent inhibition of the BCR-ABL protein, and was initially approved in 2007 by the US FDA to treat CML in patients who were either resistant to or intolerant of imatinib. A second new TK inhibitor, nilotinib, was also approved by the FDA for the same indication. In 2010, nilotinib and dasatinib were also approved for first-line therapy, making three drugs in this class available for treatment of newly diagnosed CML. In 2012, Radotinib joined the class of novel agents in the inhibition of the BCR-ABL protein and was approved in South Korea for patients resistant to or intolerant of imatinib. Bosutinib received US FDA and EU European Medicines Agency approval on September 4, 2012 and 27 March 2013 respectively for the treatment of adult patients with Philadelphia chromosome-positive (Ph+) chronic myelogenous leukemia (CML) with resistance, or intolerance to prior therapy.

Treatment-resistant CML

While capable of producing significantly improved responses compared with the action of imatinib, neither dasatinib nor nilotinib could overcome drug resistance caused by one particular mutation found to occur in the structure of BCR-ABL1 known as the T315I mutation (i.e. where the 315th amino acid is mutated from a threonine residue to an isoleucine residue). Two approaches were developed to the treatment of CML as a result:

In 2007, Chemgenex released results of an open-label Phase 2/3 study (CGX-635-CML-202) that investigated the use of a non BCR-ABL targeted agent omacetaxine, administered subcutaneously (under the skin) in patients who had failed with imatinib and exhibited T315I kinase domain mutation. This is a study which is ongoing through 2014. In September 2012, the FDA approved omacetaxine for the treatment of CML in the case of resistance to other chemotherapeutic agents.

Independently, ARIAD pharmaceuticals, adapting the chemical structures from first and second-generation TK inhibitors, arrived at a new pan-BCR-ABL1 inhibitor which showed (for the first time) efficacy against T315I, as well as all other known mutations of the oncoprotein. The drug, ponatinib, gained FDA approval in December 2012 for treatment of patients with resistant or intolerant CML. Just as with second generation TK inhibitors, early approval is being sought to extend the use of ponatinib to newly diagnosed CML also.

Vaccination

In 2005, encouraging but mixed results of vaccination were reported with the BCR/ABL1 p210 fusion protein in patients with

stable disease, with GM-CSF as an adjuvant.

Prognosis

Before the advent of tyrosine kinase inhibitors, the median survival time for CML patients had been about 3–5 years from time of diagnosis.

With the use of tyrosine kinase inhibitors, survival rates have improved dramatically. A 2006 followup of 553 patients using imatinib (Gleevec) found an overall survival rate of 89% after five years.

A 2011 followup of 832 patients using imatinib who achieved a stable cytogenetic response found an overall survival rate of 95.2% after 8 years, which is similar to the rate in the general population. Less than 1% of patients died because of leukemia progression.

Polycythemia vera

Polycythemia vera (PV) is associated most often with the JAK2 V617F mutation in greater than 95% of cases, whereas the remainder have a JAK2 exon 12 mutation:

Cellular phase - increased megakaryocytes which cluster, reticulin fibrosis, later trichrome fibrosis, and increased myeloid and erythroid precursors

Fibrotic phase - collagenous fibrosis with lack of marrow elements.

Primary myelofibrosis

Primary myelofibrosis (PMF) is associated with the JAK2V617F mutation in up to 50% of cases, the JAK2 exon 12 mutations in 1-2% of cases, and the MPL (thrombopoietin receptor) mutation in up to 5% of cases:

Prefibrotic/cellular phase - increased, small and atypical megakaryocytes which cluster, reticulin fibrosis, later trichrome (collagenous) fibrosis, and increased myeloid precursors

Fibrotic phase - collagenous fibrosis with lack of marrow elements.

Treatment

While investigational drug therapies exist, no curative drug treatment exists for any of the MPDs. The goal of treatment for ET and PV is prevention of thrombohemorrhagic complications. The goal of treatment for MF is amelioration of anemia, splenomegaly, and other symptoms. Low-dose aspirin is effective in PV and ET. Tyrosine kinase inhibitors like imatinib have improved the prognosis of CML patients to near-normal life expectancy.

Recently, a JAK2 inhibitor, namely ruxolitinib, has been approved for use in primary myelofibrosis. Trials of these inhibitors are in progress for the treatment of the other myeloproliferative neoplasms.

Thrombocythemia

Thrombocythemia (also thrombocytosis) is the presence of high platelet (thrombocyte) counts in the blood, and can be

either primary (also termed essential thrombocythemia, and caused by a myeloproliferative disease) or secondary (also termed reactive). Although often symptomless (particularly when it is a secondary reaction), it can predispose to thrombosis in some patients. Thrombocytosis can be contrasted with thrombocytopenia, a loss of platelets in the blood.

In a healthy individual, a normal platelet count ranges from 150,000 and 450,000 per mm^3 (or microlitre) (150–450 x 109/L). These limits, however, are determined by the 2.5th lower and upper percentile, and a deviation does not necessary imply any form of disease. Nevertheless, counts over 750,000 (and especially over a million) are considered serious enough to warrant investigation and intervention.

Signs and symptoms

High platelet levels do not necessarily signal any clinical problems, and are picked up on a routine full blood count. However, it is important that a full medical history be elicited to ensure that the increased platelet count is not due to a secondary process. Often, it occurs in tandem with an inflammatory disease, as the principal stimulants of platelet production (e.g. thrombopoietin) are elevated in these clinical states as part of the acute phase reaction.

High platelet counts can occur in patients with polycythemia Vera (high red blood cell counts), and is an additional risk factor for complications.

A very small segment of patients report symptoms of erythromelalgia, a burning sensation and redness of the extremities that resolves with cooling and/or aspirin use (8).

Scientific literature sometimes excludes thrombocytosis from the scope of thrombophilia by definition, but practically, by the definition of thrombophilia as an increased predisposition to thrombosis, thrombocytosis (especially primary thrombocytosis) is a potential cause of thrombophilia. Conversely, secondary thrombocytosis very rarely causes thrombotic complications.

Causes

Reactive thrombocythemia is the common cause of high platelet count. It accounts for 88% to 97% of thrombocythemia cases in adults, and 100% in children. In adults, acute infection, tissue damage, chronic inflammation and malignancy are the common causes of reactive thrombocythemia. Usually, one or more of these conditions is present in more than 75% of the cases with reactive thrombocythemia. Causes for reactive thrombocytopenia in children are similar to adults. In addition to that, haemolytic anemia and thalassemia are often present in children living in the Middle Eastern countries. Other causes of reactive thrombocythemia includes: post-surgery, iron deficiency, drugs, and rebound effect after bone marrow suppression (9).

Once the reactive causes of thrombocythemia are ruled out, clonal thrombocythemia should be considered. The most common cause of clonal thrombocythemia is myeloproliferative disease. These includes: essential thrombocythemia, chronic myelogenous leukemia, and polycythemia Vera, and primary myelofibrosis.

Extremely rare causes of thrombocythemia are spurious causes. This is due to the presence of structures resembling platelets in the blood such as needle-like cryoglobulin crystals, cytoplasmic fragments of circulating leukemic cells, bacteria, and red blood cell microvesicles. These structures are counted as platelets by the automated machine counter; therefore, causing the platelet number to be falsely elevated. However, such error can be avoided by doing a peripheral blood smear.

Diagnosis

Laboratory tests might include: full blood count, liver enzymes, renal function and erythrocyte sedimentation rate.

If the cause for the high platelet count remains unclear, bone marrow biopsy is often undertaken, to differentiate whether the high platelet count is reactive or essential.

Chronic lymphocytic leukemia

Chronic lymphocytic leukemia (CLL) is a type of cancer in which the bone marrow makes too many lymphocytes (a type of white blood cell). Early on there are typically no symptoms. Later non-painful lymph nodes swelling, feeling tired, fever, or weight loss for no clear reason may occur.Enlargement of the spleen and a low red blood cells (anemia) may also occur. It typically worsens gradually.

Risk factors include having a family history of the disease. Exposure to Agent Orange and certain insecticides might also be a risk. CLL results in the buildup of B cell lymphocytes in the bone marrow, lymph nodes, and blood. These cells do not function well and crowd out healthy blood cells. CLL is divided into two main types: those with a mutated IGHV gene and those without. Diagnosis is typically based on blood tests finding high numbers of mature lymphocytes and smudge cells.

Management of early disease is generally with watchful waiting. Infections should more readily be treated with antibiotics. In those with significant symptoms, chemotherapy or immunotherapy may be used. As of 2019 ibrutinib is often the initial medication recommended. The medications fludarabine, cyclophosphamide, and rituximab were previously the initial treatment in those who are otherwise healthy.

CLL affected about 904,000 people globally in 2015 and resulted in 60,700 deaths. The disease most commonly occurs in people over the age of 50. Males are affected more often than females. It is much less common in people from Asia. Five-year survival fol-

lowing diagnosis is approximately 83% in the United States. It represents less than 1% of deaths from cancer.

Signs and symptoms

Most people are diagnosed as having CLL based on the result of a routine blood test that shows a high white blood cell count, specifically a large increase in the number of circulating lymphocytes. These people generally have no symptoms. Less commonly, CLL may present with enlarged lymph nodes without a high white blood cell count or no evidence of the disease in the blood. This is referred to as small lymphocytic lymphoma. In some individuals, the disease comes to light only after the cancerous cells overwhelm the bone marrow resulting in anemia producing tiredness or weakness.

CLL is, in virtually all cases, preceded by a particular subtype of monoclonal B-cell lymphocytosis (MBL). This subtype, termed chronic lymphocytic leukemia/small lymphocyte lymphoma MBL (CLL/SLL MBL) is an asymptomatic, indolent, and chronic disorder in which individuals exhibit an increase in the number of circulating B-cell lymphocytes. These B-cells are abnormal: they are monoclonal, i.e. produced by a single ancestral B-cell, and have some of the same cell marker proteins, chromosome abnormalities, and gene mutations found in CLL. CLL/SLL MBL consist of two groups: low-count CLL/SLL MBL has monoclonal B-cell blood counts of <0.5x9 cells/liter (i.e. 0.5x9/L) while high-count CLL/SLL MBL has blood monoclonal B-cell counts ≥0.5x9/L but <5x109/L. Individuals with blood counts of these monoclonal B-cells >5x9/L are diagnosed as having CLL. Low-count CLL/SLLL MBL rarely if ever progresses to CLL while high-count CLL/SLL MBL does so at a rate of 1-2% per year. Thus, CLL may present in individuals with a long history of having high-count CLL/SLL MLB. There is no established treatment for these individuals except monitoring for development of the disorder's various complications (see treatment of MBL complications) and for their progression to CLL.

Complications

Complications include a low level of antibodies in the bloodstream (hypogammaglobulinemia) leading to recurrent infection, warm autoimmune hemolytic anemia in 10–15% of patients, and bone marrow failure. Chronic lymphocytic leukemia may also transform into Richter's syndrome, the development of fast-growing diffuse large B cell lymphoma, prolymphocytic leukemia, Hodgkin's lymphoma, or acute leukemia in some patients. Its incidence is estimated to be around 5% in patients with CLL.

Gastrointestinal (GI) involvement can rarely occur with chronic lymphocytic leukemia. Some of the reported manifestations include intussusception, small intestinal bacterial contamination, colitis, and others. Usually, GI complications with CLL occur after Richter transformation. Two cases to date have been reported of GI involvement in chron-

ic lymphocytic leukemia without Richter's transformation.

Causes

CLL is caused by multiple genetic mutations and epigenetic changes. Men are about twice as likely to get CLL as women, and risk increases with age. It is relatively rare among Asians. Some relevant genetic mutations may be inherited; in around 9% of CLL cases a parent had CLL. Exposure to Agent Orange increases the risk of CLL, and exposure to hepatitis C virus may increase the risk. There is no clear association between ionizing radiation exposure and the risk of developing CLL. Blood transfusions have been ruled out as a risk factor.

Diagnosis

CLL is usually first suspected by a diagnosis of lymphocytosis, an increase in a type of white blood cell, on a complete blood count test. This frequently is an incidental finding on a routine physician visit. Most often the lymphocyte count is greater than 5000 cells per microliter (μl) of blood, but can be much higher. The presence of lymphocytosis in an elderly individual should raise strong suspicion for CLL, and a confirmatory diagnostic test, in particular flow cytometry, should be performed unless clinically unnecessary.

A peripheral blood smear showing an abundance of damaged cells known as "smudge cells" can also indicate the presence of the disease (smudge cells are due to cancer cells lacking in vimentin, a cytoskeletal protein). The diagnosis of CLL is based on the demonstration of an abnormal population of B lymphocytes in the blood, bone marrow, or tissues that display an unusual but characteristic pattern of molecules on the cell surface. This atypical molecular pattern includes the coexpression of cell surface markers clusters of differentiation 5 (CD5) and 23. In addition, all the CLL cells within one individual are clonal, that is, genetically identical. In practice, this is inferred by the detection of only one of the mutually exclusive antibody light chains, kappa or lambda, on the entire population of the abnormal B cells. Normal B lymphocytes consist of a stew of different antibody-producing cells, resulting in a mixture of both kappa- and lambda-expressing cells. The lack of the normal distribution of these B cells is one basis for demonstrating clonality, the key element for establishing a diagnosis of any B cell malignancy (B cell non-Hodgkin lymphoma(.

The combination of the microscopic examination of the peripheral blood and analysis of the lymphocytes by flow cytometry to confirm clonality and marker molecule expression is needed to establish the diagnosis of CLL. Both are easily accomplished on a small amount of blood. A flow cytometer instrument can examine the expression of molecules on individual cells in fluids. This requires the use of specific antibodies to marker molecules with fluorescent tags recognized by the instrument. In CLL, the lymphocytes are genetically clonal, of the B cell lineage (expressing marker molecules

clusters of differentiation 19 and 20), and characteristically express the marker molecules CD5 and CD23. These B cells resemble normal lymphocytes under the microscope, although slightly smaller, and are fragile when smeared onto a glass slide, giving rise to many broken cells, which are called "smudge" or "smear" cells.

The Matutes's CLL score allows the identification of a homogeneous subgroup of classical CLL, that differs from atypical/mixed CLL for the five markers' expression (CD5, CD23, FMC7, CD22, and immunoglobulin light chain) Matutes's CLL scoring system is very helpful for the differential diagnosis between classical CLL and the other B cell chronic lymphoproliferative disorders, but not for the immunological distinction between mixed/atypical CLL and mantle cell lymphoma (MCL malignant B cells). Discrimination between CLL and MCL can be improved by adding non-routine markers such as CD54 and CD200. Among routine markers, the most discriminating feature is the CD20/CD23 mean fluorescence intensity ratio. In contrast, FMC7 expression can surprisingly be misleading for borderline cases.

Clinical staging

Staging, determining the extent of the disease, is done with the Rai staging system or the Binet classification and is based primarily on the presence of a low platelet or red cell count. Early-stage disease does not need to be treated. CLL and SLL are considered the same underlying disease, just with different appearances.

Rai staging system

Stage 0: characterized by absolute lymphocytosis (>15,000/mm3) without lymphadenopathy, hepatosplenomegaly, anemia, or thrombocytopenia

Stage I: characterized by absolute lymphocytosis with lymphadenopathy without hepatosplenomegaly, anemia, or thrombocytopenia

Stage II: characterized by absolute lymphocytosis with either hepatomegaly or splenomegaly with or without lymphadenopathy

Stage III: characterized by absolute lymphocytosis and anemia (hemoglobin <11 g/dL) with or without lymphadenopathy, hepatomegaly, or splenomegaly

Stage IV: characterized by absolute lymphocytosis and thrombocytopenia (<100,000/mm3) with or without lymphadenopathy, hepatomegaly, splenomegaly, or anemia

Binet classification

Clinical stage A: characterized by no anemia or thrombocytopenia and fewer than three areas of lymphoid involvement (Rai stages 0, I, and II(

Clinical stage B: characterized by no anemia or thrombocytopenia with three or more areas of lymphoid involvement (Rai stages I and II(

Clinical stage C: characterized by anemia

and/or thrombocytopenia regardless of the number of areas of lymphoid enlargement (Rai stages III and IV).

Array-based karyotyping

Virtual karyotype

Array-based karyotyping is a cost-effective alternative to FISH for detecting chromosomal abnormalities in CLL. Several clinical validation studies have shown >95% concordance with the standard CLL FISH panel.

Related diseases

In the past, cases with similar microscopic appearance in the blood but with a T cell phenotype were referred to as T-cell CLL. However, these are now recognized as a separate disease group and are currently classified as T-cell prolymphocytic leukemias.

CLL should not be confused with acute lymphoblastic leukemia, a highly aggressive leukemia most commonly diagnosed in children, and highly treatable in the pediatric setting.

Differential diagnosis

Lymphoid disorders that can present as chronic leukemia and can be confused with typical B-cell chronic lymphoid leukemia.

Follicular lymphoma

Splenic marginal zone lymphoma

Nodal marginal zone lymphoma

Mantle cell lymphoma

Hairy cell leukemia

Prolymphocytic leukemia (B cell or T cell)

Lymphoplasmacytic lymphoma

Sézary syndrome

Smoldering adult T cell leukemia/lymphoma

Hematologic disorders that may resemble CLL in their clinical presentation, behavior, and microscopic appearance include mantle cell lymphoma, marginal zone lymphoma, B cell prolymphocytic leukemia, and lymphoplasmacytic lymphoma.

B cell prolymphocytic leukemia, a related, but more aggressive disorder, has cells with similar phenotype, but are significantly larger than normal lymphocytes and have a prominent nucleolus. The distinction is important as the prognosis and therapy differ from CLL.

Hairy cell leukemia is also a neoplasm of B lymphocytes, but the neoplastic cells have a distinct morphology under the microscope (hairy cell leukemia cells have delicate, hair-like projections on their surfaces) and unique marker molecule expression.

All the B cell malignancies of the blood and bone marrow can be differentiated from one another by the combination of cellular microscopic morphology, marker molecule expression, and specific tumor-associated gene defects. This is best accomplished by evaluation of the patient's blood, bone mar-

row, and occasionally lymph node cells by a pathologist with specific training in blood disorders. A flow cytometer is necessary for cell marker analysis, and the detection of genetic problems in the cells may require visualizing the DNA changes with fluorescent probes by FISH.

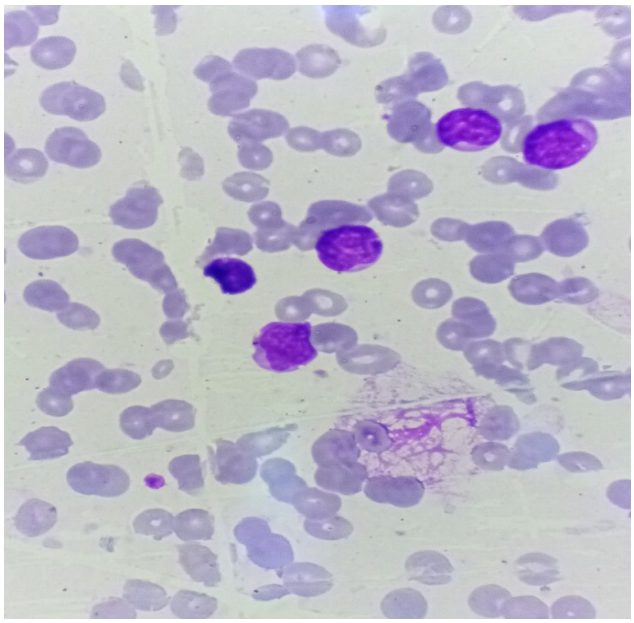

Figure (4-4) Blood film stained by Romanowsky stain shows mature appearing lymphocytes and many smear cell in patient with CLL.

Prognosis

Prognosis depends on the subtype. Some subtypes have a median survival of 6–8 years, while others have a median survival of 22 years (which is a normal lifespan for older patients). Telomere length has been suggested to be a valuable prognostic indicator of survival.

Epidemiology

CLL is primarily a disease of older adults, with a median age of 70 years at the time of diagnosis. Though less common, CLL sometimes affects people between 30 and 39 years of age. The incidence of CLL increases very quickly with increasing age.

In the United States during 2014, about 15,720 new cases are expected to be diagnosed, and 4,600 patients are expected to die from CLL. Because of the prolonged survival, which was typically about 10 years in past decades, but which can extend to a normal life expectancy, the prevalence (number of people living with the disease) is much higher than the incidence (new diagnoses). CLL is the most common type of leukemia in the UK, accounting for 38% of all leukemia cases. Approximately 3,200 people were diagnosed with the disease in 2011.

In Western populations, subclinical "disease" can be identified in 3.5% of normal adults, and in up to 8% of individuals over the age of 70. That is, small clones of B cells with the characteristic CLL phenotype can be identified in many healthy elderly persons. The clinical significance of these cells is unknown.

In contrast, CLL is rare in Asian countries, such as Japan, China, and Korea, accounting for less than 10% of all leukemias in those regions. A low incidence is seen in Japanese immigrants to the US, and in African and Asian immigrants to Israel.

Of all cancers involving the same class of blood cell, 7% of cases are CLL/SLL.

Rates of CLL are somewhat elevated in peo-

ple exposed to certain chemicals. Under U.S. Department of Veterans' Affairs regulations, Vietnam veterans who served in-country or in the inland waterways of Vietnam and who later develop CLL are presumed to have contracted it from exposure to Agent Orange and may be entitled to compensation.

Treatment

CLL treatment focuses on controlling the disease and its symptoms rather than on an outright cure. In those without or only minimal symptoms watchful waiting is generally appropriate.

CLL is treated by chemotherapy, radiation therapy, biological therapy, or bone marrow transplantation. Symptoms are sometimes treated surgically (splenectomy – removal of enlarged spleen) or by radiation therapy ("de-bulking" swollen lymph nodes(.

Initial CLL treatments vary depending on the exact diagnosis and the progression of the disease, and even with the preference and experience of the health care practitioner. Any of dozens of agents may be used for CLL therapy.

Decision to treat

While it is generally considered incurable, CLL progresses slowly in most cases. Many people with CLL lead normal and active lives for many years—in some cases for decades. Because of its slow onset, early-stage CLL is, in general, not treated since it is believed that early CLL intervention does not improve survival time or quality of life. Instead, the condition is monitored over time to detect any change in the disease pattern.

The decision to start CLL treatment is taken when the person's symptoms or blood counts indicate that the disease has progressed to a point where it may affect quality of life.

Clinical "staging systems" such as the Rai four-stage system and the Binet classification can help to determine when and how to treat the patient.

Determining when to start treatment and by what means is often difficult; no survival advantage is seen in treating the disease very early. The National Cancer Institute Working Group has issued guidelines for treatment, with specific markers that should be met before it is initiated.

Chemotherapy

Combination chemotherapy regimens are effective in both newly diagnosed and relapsed CLL. Combinations of fludarabine with alkylating agents (cyclophosphamide) produce higher response rates and a longer progression-free survival than single agents:

FC (fludarabine with cyclophosphamide)

FR (fludarabine with rituximab)

FCR (fludarabine, cyclophosphamide, and rituximab)

CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisolone(

Although the purine analogue fludarabine was shown to give superior response rates to chlorambucil as primary therapy, no evidence shows early use of fludarabine improves overall survival, and some clinicians prefer to reserve fludarabine for relapsed disease.

Chemoimmunotherapy with FCR has shown to improve response rates, progression-free survival, and overall survival in a large randomized trial in CLL patients selected for good physical fitness. This has been the first clinical trial demonstrating that the choice of a first-line therapy can improve the overall survival of patients with CLL.

Alkylating agents approved for CLL include bendamustine and cyclophosphamide. Targeted therapy

Targeted therapy attacks cancer cells at a specific target, with the aim of not harming normal cells. Targeted drugs used in CLL include venetoclax (a Bcl-2 inhibitor), ibrutinib (a Bruton's tyrosine kinase inhibitor), idelalisib and duvelisib (inhibitors of some forms of the enzyme phosphoinositide 3-kinase), as well as monoclonal antibodies against CD20 (rituximab, ofatumumab and obinutuzumab) and CD52 (alemtuzumab).

Stem cell transplantation

Autologous stem cell transplantation, using the recipient's own cells, is not curative. Younger individuals, if at high risk for dying from CLL, may consider allogeneic hematopoietic stem cell transplantation (HSCT). Myeloablative (bone marrow killing) forms of allogeneic stem cell transplantation, a high-risk treatment using blood cells from a healthy donor, may be curative, but treatment-related toxicity is significant. An intermediate level, called reduced-intensity conditioning allogeneic stem cell transplantation, may be better tolerated by older or frail patients.

Refractory CLL

"Refractory" CLL is a disease that no longer responds favorably to treatment. In this case, more aggressive therapies, including lenalidomide, flavopiridol, and bone marrow (stem cell) transplantation, are considered. The monoclonal antibody alemtuzumab (directed against CD52) may be used in patients with refractory, bone marrow-based disease.

During pregnancy

Leukemia is rarely associated with pregnancy, affecting only about one in 10,000 pregnant women. Treatment for chronic lymphocytic leukemias can often be postponed until after the end of the pregnancy. If treatment is necessary, then giving chemotherapy during the second or third trimesters is less likely to result in pregnancy loss or birth defects than treatment during the first trimester

Lymphoproliferative disorders

Lymphoproliferative disorders (LPDs) refer

to several conditions in which lymphocytes are produced in excessive quantities. They typically occur in people who have a compromised immune system. They are sometimes equated with "immunoproliferative disorders", but technically lymphoproliferative disorders are a subset of immunoproliferative disorders, along with hypergammaglobulinemia and paraproteinemias.

EXAMPLE

follicular lymphoma

chronic lymphocytic leukemia

acute lymphoblastic leukemia

hairy cell leukemia

B-cell lymphomas

T-cell lymphomas

multiple myeloma

Waldenstrom's macroglobulinemia

Wiskott-Aldrich syndrome

Lymphocyte-variant hypereosinophilia

Pityriasis Lichenoides (PL, PLC, PLVA)

post-transplant lymphoproliferative disorder

autoimmune lymphoproliferative syndrome (ALPS)

Lymphoid interstitial pneumonia"[1]

Epstein-Barr virus-associated lymphoproliferative diseases

Types

Lymphoproliferative disorders are a set of disorders characterized by the abnormal proliferation of lymphocytes into a monoclonal lymphocytosis. The two major types of lymphocytes are B cells and T cells, which are derived from pluripotent hematopoetic stem cells in the bone marrow. Individuals who have some sort of dysfunction with their immune system are susceptible to develop a lymphoproliferative disorder because when any of the numerous control points of the immune system become dysfunctional, immunodeficiency or deregulation of lymphocytes is more likely to occur. There are several inherited gene mutations that have been identified to cause lymphoproliferative disorders; however, there are also acquired and iatrogenic causes.

X-linked Lymphoproliferative disorder

A mutation on the X chromosome is associated with a T cell and natural killer cell lymphoproliferative disorder.

Autoimmune lymphoproliferative syndrome

Some children with autoimmune lymphoproliferative disorders are heterozygous for a mutation in the gene that codes for the Fas receptor, which is located on the long arm of chromosome 10 at position 24.1, denoted 10q24.1 This gene is member 6 of the TNF-receptor superfamily (TNFRSF6). The

Fas receptor contains a death domain and has been shown to play a central role in the physiological regulation of programmed cell death. Normally, stimulation of recently activated T cells by antigen leads to coexpression of Fas and Fas receptor on the T cell surface. The engagement of Fas by Fas receptor results in apoptosis of the cell and is important for eliminating T cells that are repeatedly stimulated by antigens. As a result of the mutation in the Fas receptor gene, there is no recognition of Fas by Fas receptor, leading to a primitive population of T cells that proliferates in an uncontrolled manner.

Other inherited causes

Boys with X-linked immunodeficiency syndrome are at a higher risk of mortality associated with Epstein - Barr virus infections, and are predisposed to develop a lymphoproliferative disorder or lymphoma.

Children with common variable immune deficiency (CVID) are also at a higher risk of developing a lymphoproliferative disorder.

Some disorders that predispose a person to lymphoproliferative disorders are severe combined immuno deficiency (SCID), Chédiak-Higashi syndrome, Wiskott-Aldrich syndrome (an X-linked recessive disorder), and ataxia telangiectasia.

Even though ataxia telangiectasia is an autosomal recessive disorder, people who are heterozygotes for this still have an increased risk of developing a lymphoproliferative disorder.

Acquired causes

Viral infection is a very common cause of lymphoproliferative disorders. In children, the most common is believed to be congenital HIV infection because it is highly associated with acquired immunodeficiency, which often leads to lymphoproliferative disorders.

Iatrogenic causes

There are many lymphoproliferative disorders that are associated with organ transplantation and immunosuppressant therapies. In most reported cases, these cause B cell lymphoproliferative disorders; however, some T cell variations have been described. The T cell variations are usually caused by the prolonged use of T cell suppressant drugs, such as sirolimus, tacrolimus, or ciclosporin. The Epstein-Barr virus, which infects >90% of the world population, is also a common cause of these disorders, being responsible for a wide range of non-malignant, pre-malignant, and malignant Epstein-Barr virus-associated lymphoproliferative diseases.

Hodgkin's lymphoma

Hodgkin's lymphoma (HL) is a type of lymphoma in which cancer originates from a specific type of white blood cells called lymphocytes. Symptoms may include fever, night sweats, and weight loss. Often there will be non-painful enlarged lymph nodes in the neck, under the arm, or in the groin.

Those affected may feel tired or be itchy.

About half of cases of Hodgkin's lymphoma are due to Epstein–Barr virus (EBV). Other risk factors include a family history of the condition and having HIV/AIDS. There are two major types of Hodgkin lymphoma: classical Hodgkin lymphoma and nodular lymphocyte-predominant Hodgkin lymphoma. Diagnosis is by finding Hodgkin's cells such as multinucleated Reed–Sternberg cells (RS cells) in lymph nodes. The virus-positive cases are classified as a form of the Epstein-Barr virus-associated lymphoproliferative diseases.

Hodgkin lymphoma may be treated with chemotherapy, radiation therapy, and stem cell transplant. The choice of treatment often depends on how advanced the cancer has become and whether or not it has favorable features. In early disease, a cure is often possible. The percentage of people who survive five years in the United States is 86%. For those under the age of 20, rates of survival are 97%. Radiation and some chemotherapy drugs, however, increase the risk of other cancers, heart disease, or lung disease over the subsequent decades.

In 2015, about 574,000 people had Hodgkin's lymphoma, and 23,900 died. In the United States, 0.2% of people are affected at some point in their life. The most common age of diagnosis is between 20 and 40 years old. It was named after the English physician Thomas Hodgkin, who first described the condition in 1832.

Signs and symptoms

People with Hodgkin's lymphoma may present with the following symptoms:

Lymph nodes: the most common symptom of Hodgkin's is the painless enlargement of one or more lymph nodes, or lymphadenopathy. The nodes may also feel rubbery and swollen when examined. The nodes of the neck and shoulders (cervical and supraclavicular) are most frequently involved (80–90% of the time, on average). The lymph nodes of the chest are often affected, and these may be noticed on a chest radiograph.

Itchy skin

Night sweats

Unexplained weight loss

Splenomegaly: enlargement of the spleen occurs in about 30% of people with Hodgkin's lymphoma. The enlargement, however, is seldom massive, and the size of the spleen may fluctuate during the course of treatment.

Hepatomegaly: enlargement of the liver, due to liver involvement, is present in about 5% of cases.

Hepatosplenomegaly: the enlargement of both the liver and spleen caused by the same disease.

Pain following alcohol consumption: classically, involved nodes are painful after alcohol consumption, though this phenomenon is very uncommon, occurring in only two to

three percent of people with Hodgkin's lymphoma, thus having a low sensitivity. On the other hand, its positive predictive value is high enough for it to be regarded as a pathognomonic sign of Hodgkin's lymphoma. The pain typically has an onset within minutes after ingesting alcohol, and is usually felt as coming from the vicinity where there is an involved lymph node. The pain has been described as either sharp and stabbing or dull and aching.

Back pain: nonspecific back pain (pain that cannot be localised or its cause determined by examination or scanning techniques) has been reported in some cases of Hodgkin's lymphoma. The lower back is most often affected.

Red-coloured patches on the skin, easy bleeding and petechiae due to low platelet count (as a result of bone marrow infiltration, increased trapping in the spleen etc.—i.e. decreased production, increased removal(

Systemic symptoms: about one-third of people with Hodgkin's disease may also present with systemic symptoms, including low-grade fever; night sweats; unexplained weight loss of at least 10% of the person's total body mass in six months or less, itchy skin (pruritus) due to increased levels of eosinophils in the bloodstream; or fatigue (lassitude). Systemic symptoms such as fever, night sweats, and weight loss are known as B symptoms; thus, presence of fever, weight loss, and night sweats indicate that the person's stage is, for example, 2B instead of 2A.

Cyclical fever: people may also present with a cyclical high-grade fever known as the Pel–Ebstein fever, or more simply "P-E fever". However, there is debate as to whether the P-E fever truly exists.

Nephrotic syndrome can occur in individuals with Hodgkin's lymphoma and is most commonly caused by minimal change disease.

Diagnosis

Hodgkin's lymphoma must be distinguished from non-cancerous causes of lymph node swelling (such as various infections) and from other types of cancer. Definitive diagnosis is by lymph node biopsy (usually excisional biopsy with microscopic examination). Blood tests are also performed to assess function of major organs and to assess safety for chemotherapy. Positron emission tomography (PET) is used to detect small deposits that do not show on CT scanning. PET scans are also useful in functional imaging (by using a radiolabeled glucose to image tissues of high metabolism). In some cases a Gallium scan may be used instead of a PET scan.

Types

Classical Hodgkin lymphoma (excluding nodular lymphocyte predominant Hodgkin's lymphoma) can be subclassified into four pathologic subtypes based upon Reed–Sternberg cell morphology and the composi-

tion of the reactive cell infiltrate seen in the lymph node biopsy specimen (the cell composition around the Reed–Sternberg cells (

Nodular lymphocyte predominant Hodgkin's lymphoma expresses CD20, and is not currently considered a form of classical Hodgkin's lymphoma.

For the other forms, although the traditional B-cell markers (such as CD20) are not expressed on all cells, Reed–Sternberg cells are usually of B cell origin. Although Hodgkin's is now frequently grouped with other B-cell malignancies, some T-cell markers (such as CD2 and CD4) are occasionally expressed. However, this may be an artifact of the ambiguity inherent in the diagnosis.

Hodgkin cells produce interleukin-21 (IL-21), which was once thought to be exclusive to T-cells. This feature may explain the behavior of classical Hodgkin's lymphoma, including clusters of other immune cells gathered around HL cells (infiltrate) in cultures.

Staging

The staging is the same for both Hodgkin's and non-Hodgkin's lymphomas.

After Hodgkin lymphoma is diagnosed, a person will be staged: that is, they will undergo a series of tests and procedures that will determine what areas of the body are affected. These procedures may include documentation of their histology, a physical examination, blood tests, chest X-ray radiographs, computed tomography (CT)/ Positron emission tomography (PET)/magnetic resonance imaging (MRI) scans of the chest, abdomen and pelvis, and usually a bone marrow biopsy. Positron emission tomography (PET) scan is now used instead of the gallium scan for staging. On the PET scan, sites involved with lymphoma light up very brightly enabling accurate and reproducible imaging. In the past, a lymphangiogram or surgical laparotomy (which involves opening the abdominal cavity and visually inspecting for tumors) were performed. Lymphangiograms or laparotomies are very rarely performed, having been supplanted by improvements in imaging with the CT scan and PET scan.

On the basis of this staging, the person will be classified according to a staging classification (the Ann Arbor staging classification scheme is a common one:

Stage I is involvement of a single lymph node region (I) (mostly the cervical region) or single extralymphatic site (Ie(

Stage II is involvement of two or more lymph node regions on the same side of the diaphragm (II) or of one lymph node region and a contiguous extralymphatic site (IIe(

Stage III is involvement of lymph node regions on both sides of the diaphragm, which may include the spleen (IIIs) or limited contiguous extralymphatic organ or site (IIIe, IIIes(

Stage IV is disseminated involvement of one or more extralymphatic organs.

The absence of systemic symptoms is signified by adding "A" to the stage; the presence of systemic symptoms is signified by adding "B" to the stage. For localised extranodal extension from mass of nodes that does not advance the stage, subscript "E" is added. Splenic involvement is signified by adding "S" to the stage. The inclusion of "bulky disease" is signified by "X".

Pathology

Macroscopy

Affected lymph nodes (most often, laterocervical lymph nodes) are enlarged, but their shape is preserved because the capsule is not invaded. Usually, the cut surface is white-grey and uniform; in some histological subtypes (e.g. nodular sclerosis) a nodular aspect may appear.

A fibrin ring granuloma may be seen.

Microscopic examination of the lymph node biopsy reveals complete or partial effacement of the lymph node architecture by scattered large malignant cells known as Reed-Sternberg cells (RSC) (typical and variants) admixed within a reactive cell infiltrate composed of variable proportions of lymphocytes, histiocytes, eosinophils, and plasma cells. The Reed–Sternberg cells are identified as large often bi-nucleated cells with prominent nucleoli and an unusual CD45-, CD30+, CD15+/- immunophenotype. In approximately 50% of cases, the Reed–Sternberg cells are infected by the Epstein–Barr virus.

Characteristics of classic Reed–Sternberg cells include large size (20–50 micrometres), abundant, amphophilic, finely granular/homogeneous cytoplasm; two mirror-image nuclei (owl eyes) each with an eosinophilic nucleolus and a thick nuclear membrane (chromatin is distributed close to the nuclear membrane

Variants:

Hodgkin cell (atypical mononuclear RSC) is a variant of RS cell, which has the same characteristics, but is mononucleated.

Lacunar RSC is large, with a single hyperlobulated nucleus, multiple, small nucleoli and eosinophilic cytoplasm which is retracted around the nucleus, creating an empty space ("lacunae").

Pleomorphic RSC has multiple irregular nuclei.

"Popcorn" RSC (lympho-histiocytic variant) is a small cell, with a very lobulated nucleus, small nucleoli.

"Mummy" RSC has a compact nucleus with no nucleolus and basophilic cytoplasm.

Hodgkin's lymphoma can be sub-classified by histological type. The cell histology in Hodgkin's lymphoma is not as important as it is in non-Hodgkin's lymphoma: the treatment and prognosis in classic Hodgkin's lymphoma usually depends on the stage of disease rather than the histotype.

Management

People with early stage disease (IA or IIA) are effectively treated with radiation therapy or chemotherapy. The choice of treatment depends on the age, sex, bulk and the histological subtype of the disease. Adding localised radiation therapy after the chemotherapy regimen may provide a longer progression-free survival compared with chemotherapy treatment alone. People with later disease (III, IVA, or IVB) are treated with combination chemotherapy alone. People of any stage with a large mass in the chest are usually treated with combined chemotherapy and radiation therapy.

The common non-Hodgkin's treatment, rituximab (which is a monoclonal antibody against CD20) is not routinely used to treat Hodgkin's lymphoma due to the lack of CD20 surface antigens in most cases. The use of rituximab in Hodgkin's lymphoma, including the lymphocyte predominant subtype has been recently reviewed.

Although increased age is an adverse risk factor for Hodgkin's lymphoma, in general elderly people without major comorbidities are sufficiently fit to tolerate standard therapy, and have a treatment outcome comparable to that of younger people. However, the disease is a different entity in older people and different considerations enter into treatment decisions.

For Hodgkin's lymphomas, radiation oncologists typically use external beam radiation therapy (sometimes shortened to EBRT or XRT). Radiation oncologists deliver external beam radiation therapy to the lymphoma from a machine called a linear accelerator which produces high energy X-rays and electrons. People usually describe treatments as painless and similar to getting an X-ray. Treatments last less than 30 minutes each.

For lymphomas, there are a few different ways radiation oncologists target the cancer cells. Involved site radiation is when the radiation oncologists give radiation only to those parts of the person's body known to have the cancer. Very often, this is combined with chemotherapy. Radiation therapy directed above the diaphragm to the neck, chest or underarms is called mantle field radiation. Radiation to below the diaphragm to the abdomen, spleen or pelvis is called inverted-Y field radiation. Total nodal irradiation is when the therapist gives radiation to all the lymph nodes in the body to destroy cells that may have spread.

Adverse effects

The high cure rates and long survival of many people with Hodgkin's lymphoma has led to a high concern with late adverse effects of treatment, including cardiovascular disease and second malignancies such as acute leukemias, lymphomas, and solid tumors within the radiation therapy field. Most people with early-stage disease are now treated with abbreviated chemotherapy and involved site radiation therapy rather than with radiation therapy alone. Clinical research strategies

are exploring reduction of the duration of chemotherapy and dose and volume of radiation therapy in an attempt to reduce late morbidity and mortality of treatment while maintaining high cure rates. Hospitals are also treating those who respond quickly to chemotherapy with no radiation.

In childhood cases of Hodgkin's lymphoma, long-term endocrine adverse effects are a major concern, mainly gonadal dysfunction and growth retardation. Gonadal dysfunction seems to be the most severe endocrine long-term effect, especially after treatment with alkylating agents or pelvic radiotherapy.

Multiple myeloma

Multiple myeloma, also known as plasma cell myeloma, is a cancer of plasma cells, a type of white blood cell typically responsible for producing antibodies. Often, no symptoms are noticed initially. When advanced, bone pain, bleeding, frequent infections, and anemia may occur. Complications may include amyloidosis.

The cause of multiple myeloma is unknown. Risk factors include obesity, radiation exposure, family history, and certain chemicals. Multiple myeloma may develop from monoclonal gammopathy of undetermined significance that progresses to smoldering multiple myeloma. The abnormal plasma cells produce abnormal antibodies which can cause kidney problems and overly thick blood. The plasma cells can also form a mass in the bone marrow or soft tissue. When only one mass is present, it is known as a plasmacytoma, while more than one is known as multiple myeloma. Multiple myeloma is diagnosed based on blood or urine tests finding abnormal antibodies, bone marrow biopsy finding cancerous plasma cells, and medical imaging finding bone lesions. Another common finding is high blood calcium levels.

Multiple myeloma is considered treatable, but generally incurable. Remissions may be brought about with steroids, chemotherapy, targeted therapy, and stem cell transplant. Bisphosphonates and radiation therapy are sometimes used to reduce pain from bone lesions.

Globally, multiple myeloma affected 488,000 people and resulted in 101,100 deaths in 2015. In the United States, it develops in 6.5 per 100,000 people per year and 0.7% of people are affected at some point in their lives. It usually occurs around the age of 61 and is more common in men than women. It is uncommon before the age of 40.Without treatment, typical survival is seven months. With current treatments, survival is usually 4 to 5 years. This gives a five-year survive.

Signs and symptoms

Because many organs can be affected by myeloma, the symptoms and signs vary greatly. A mnemonic sometimes used to remember some of the common symptoms of multiple myeloma is CRAB: C = calcium (elevated), R

= renal failure, A = anemia, B = bone lesions. Myeloma has many other possible symptoms, including opportunistic infections (e.g., pneumonia) and weight loss. CRAB symptoms and proliferation of monoclonal plasma cells in the bone marrow are part of the diagnostic criteria of multiple myeloma.

Bone pain affects almost 70% of patients and is the most common symptom. Myeloma bone pain usually involves the spine and ribs, and worsens with activity. Persistent localized pain may indicate a pathological bone fracture. Involvement of the vertebrae may lead to spinal cord compression or kyphosis. Myeloma bone disease is due to the overexpression of receptor activator for nuclear factor κ B ligand (RANKL) by bone marrow stroma. RANKL activates osteoclasts, which resorb bone. The resultant bone lesions are lytic (cause breakdown) in nature, and are best seen in plain radiographs, which may show "punched-out" resorptive lesions (including the "raindrop" appearance of the skull on radiography). The breakdown of bone also leads to the release of calcium into the blood, leading to hypercalcemia and its associated symptoms.

Anemia

The anemia found in myeloma is usually normocytic and normochromic. It results from the replacement of normal bone marrow by infiltrating tumor cells and inhibition of normal red blood cell production (hematopoiesis) by cytokines.

Kidney failure

The most common cause of kidney failure in multiple myeloma is due to proteins secreted by the malignant cells. Myeloma cells produce monoclonal proteins of varying types, most commonly immunoglobulins (antibodies) and free light chains, resulting in abnormally high levels of these proteins in the blood. Depending on the size of these proteins, they may be excreted through the kidneys. Kidneys can be damaged by the effects of proteins or light chains. Increased bone resorption leads to hypercalcemia and causes nephrocalcinosis, thereby contributing to the kidney failure. Amyloidosis is a distant third in the causation. Patients with amyloidosis have high levels of amyloid protein that can be excreted through the kidneys and cause damage to the kidneys and other organs.

Light chains produce myriad effects which can manifest as the Fanconi syndrome (type II renal tubular acidosis).

Infection

The most common infections are pneumonias and pyelonephritis. Common pneumonia pathogens include S. pneumoniae, S. aureus, and K. pneumoniae, while common pathogens causing pyelonephritis include E. coli and other Gram-negative organisms. The greatest risk period for the occurrence of infection is in the initial few months after the start of chemotherapy. The increased risk of infection is due to immune deficien-

cy. Although the total immunoglobulin level is typically elevated in multiple myeloma, the majority of the antibodies are ineffective monoclonal antibodies from the clonal plasma cell. A selected group of patients with documented hypogammaglobulinemia may benefit from replacement immunoglobulin therapy to reduce the risk of infection.

Neurological symptoms

Some symptoms (e.g., weakness, confusion, and fatigue) may be due to anemia or hypercalcemia. Headache, visual changes, and retinopathy may be the result of hyperviscosity of the blood depending on the properties of the paraprotein. Finally, radicular pain, loss of bowel or bladder control (due to involvement of spinal cord leading to cord compression) or carpal tunnel syndrome, and other neuropathies (due to infiltration of peripheral nerves by amyloid) may occur. It may give rise to paraplegia in late-presenting cases.

When the disease is well-controlled, neurological symptoms may result from current treatments, some of which may cause peripheral neuropathy, manifesting itself as numbness or pain in the hands, feet, and lower legs.

Pathophysiology

B lymphocytes start in the bone marrow and move to the lymph nodes. As they progress, they mature and display different proteins on their cell surface. When they are activated to secrete antibodies, they are known as plasma cells.

Multiple myeloma develops in B lymphocytes after they have left the part of the lymph node known as the germinal center. The normal cell line most closely associated with MM cells is generally taken to be either an activated memory B cell or the precursor to plasma cells, the plasmablast.

The immune system keeps the proliferation of B cells and the secretion of antibodies under tight control. When chromosomes and genes are damaged, often through rearrangement, this control is lost. Often, a promoter gene moves (or translocates) to a chromosome where it stimulates an antibody gene to overproduction.

A chromosomal translocation between the immunoglobulin heavy chain gene (on chromosome 14, locus q32) and an oncogene (often 11q13, 4p16.3, 6p21, 16q23 and 20q11) is frequently observed in patients with multiple myeloma. This mutation results in dysregulation of the oncogene which is thought to be an important initiating event in the pathogenesis of myeloma. The result is a proliferation of a plasma cell clone and genomic instability that leads to further mutations and translocations. The chromosome 14 abnormality is observed in about 50% of all cases of myeloma. Deletion of (parts of) chromosome 13 is also observed in about 50% of cases.

Production of cytokines (especially IL-6) by the plasma cells causes much of their

localised damage, such as osteoporosis, and creates a microenvironment in which the malignant cells thrive. Angiogenesis (the generation of new blood vessels) is increased.

The produced antibodies are deposited in various organs, leading to kidney failure, polyneuropathy, and various other myeloma-associated symptoms.

Genetics

Mutations in a number of genes have been associated with this condition. These include ATM, BRAF, CCND1, DIS3, FAM46C, KRAS, NRAS and TP53.

Development

The genetic and epigenetic changes occur progressively. The initial change, often involving one chromosome 14 translocation, establishes a clone of bone marrow plasma cells that causes the asymptomatic disorder monoclonal gammopathy of undetermined significance (MGUS). MGUS is a premalignant disorder characterized by increased numbers of plasma cells in the bone marrow or the circulation of a myeloma protein immunoglobulin. Further genetic or epigenic changes produce a new clone of bone marrow plasma cells, usually descendant from the original clone that causes the more serious but still asymptomatic premalignant disorder smoldering multiple myeloma. Smoldering multiple myeloma is characterized by a rise in the number of bone marrow plasma cells or levels of the circulating myeloma protein above that seen in MGUS.

Subsequent genetic and epigenetic changes lead to a new, more aggressive clone of plasma cells which cause further rises in the level of the circulating myeloma protein, further rises in the number of bone marrow plasma cells, or the development of one or more of a specific set of "CRAB" symptoms which are the basis for diagnosing malignant multiple myeloma and treating the disease.

In a small percentage of multiple myeloma cases, further genetic and epigenetic changes lead to the development of a plasma cell clone that moves from the bone marrow into the circulatory system, invades distant tissues, and thereby causes the most malignant of all plasma cell dyscrasias, plasma cell leukemia. Thus, a fundamental genetic instability in plasma cells or their precursors leads to the progression:

Monoclonal gammopathy of undetermined significance → smoldering multiple myeloma → Multiple myeloma → Plasma cell leukemia

Being asymptomatic, monoclonal gammapathy of undetermined significance and smoldering multiple myeloma are typically diagnosed fortuitously by detecting a myeloma protein on serum protein electrophoresis tests done for other purposes. Monoclonal gammopathy of undetermined significance is a relatively stable condition afflicting 3% of people aged 50 and 5% of people aged 70; it progresses to multiple myeloma at a rate

of 0.5-1% cases per year; smoldering multiple myeloma does so at a rate of 10% per year for the first 5 years but then falls off sharply to 3% per year for the next 5 years and thereafter to 1% per year.

Overall, some 2-4% of multiple myeloma cases eventually progress to plasma cell leukemia.

Diagnosis

The presence of unexplained anemia, kidney dysfunction, a high erythrocyte sedimentation rate (ESR), lytic bone lesions, elevated beta-2 microglobulin, or a high serum protein (especially raised globulins or immunoglobulin) may prompt further testing.

Blood tests

The globulin level may be normal in established disease. A doctor will request protein electrophoresis of the blood and urine, which might show the presence of a paraprotein (monoclonal protein, or M protein) band, with or without reduction of the other (normal) immunoglobulins (known as immune paresis). One type of paraprotein is the Bence Jones protein which is a urinary paraprotein composed of free light chains. Quantitative measurements of the paraprotein are necessary to establish a diagnosis and to monitor the disease. The paraprotein is an abnormal immunoglobulin produced by the tumor clone.

In theory, multiple myeloma can produce all classes of immunoglobulin, but IgG paraproteins are most common, followed by IgA and IgM. IgD and IgE myeloma are very rare. In addition, light and or heavy chains (the building blocks of antibodies) may be secreted in isolation: κ- or λ-light chains or any of the five types of heavy chains (α-, γ-, δ-, ε- or μ-heavy chains). Patients without evidence of a monoclonal protein may have "nonsecretory" myeloma (not producing immunoglobulins); this represents approximately 3% of all multiple myeloma patients.

Additional findings may include: a raised calcium (when osteoclasts are breaking down bone, releasing calcium into the bloodstream), raised serum creatinine due to reduced kidney function, which is mainly due to casts of paraprotein deposition in the kidney, although the cast may also contain complete immunoglobulins, Tamm-Horsfall protein and albumin.

Other useful laboratory tests include quantitative measurement of IgA, IgG, IgM (immunoglobulins) to look for immune paresis, and beta-2 microglobulin which provides prognostic information. On peripheral blood smear, the rouleaux formation of red blood cells is commonly seen, though this is not specific.

The recent introduction of a commercial immunoassay for measurement of free light chains potentially offers an improvement in monitoring disease progression and response to treatment, particularly where the paraprotein is difficult to measure accurately by electrophoresis (for example in light

chain myeloma, or where the paraprotein level is very low). Initial research also suggests that measurement of free light chains may also be used, in conjunction with other markers, for assessment of the risk of progression from monoclonal gammopathy of undetermined significance (MGUS) to multiple myeloma.

This assay, the serum free light chain assay, has recently been recommended by the International Myeloma Working Group for the screening, diagnosis, prognosis, and monitoring of plasma cell dyscrasias.

Histopathology

A bone marrow biopsy is usually performed to estimate the percentage of bone marrow occupied by plasma cells. This percentage is used in the diagnostic criteria for myeloma. Immunohistochemistry (staining particular cell types using antibodies against surface proteins) can detect plasma cells which express immunoglobulin in the cytoplasm and occasionally on the cell surface; myeloma cells are often CD56, CD38, CD138, CD319 positive and CD19, CD20 and CD45 negative. Flow cytometry is often used to establish the clonal nature of the plasma cells, which will generally express only kappa or lambda light chain. Cytogenetics may also be performed in myeloma for prognostic purposes, including a myeloma-specific FISH and virtual karyotype.

The plasma cells seen in multiple myeloma have several possible morphologies. First, they could have the appearance of a normal plasma cell: a large cell two or three times the size of a peripheral lymphocyte. Because they are actively producing antibodies, the Golgi apparatus will typically produce a light-colored area adjacent to the nucleus, called a perinuclear halo. The single nucleus (with inside a single nucleolus with vesicular nuclear chromatin) is eccentric, displaced by an abundant cytoplasm. Other common morphologies that are seen, but which are not usual in normal plasma cells, include:

Bizarre cells, which are multinucleated.

Mott cells, containing multiple clustered cytoplasmic droplets or other inclusions (sometimes confused with auer rods, commonly seen in myeloid blasts), Flame cells, having a fiery red cytoplasm.

Historically, the CD138 has been used to isolate myeloma cells for diagnostic purposes. However, this antigen disappears rapidly ex vivo. Recently, however, it was discovered that the surface antigen CD319 (SLAMF7) is considerably more stable and allows robust isolation of malignant plasma cells from delayed or even cryopreserved samples.

The prognosis varies widely depending upon various risk factors. The Mayo Clinic has developed a risk-stratification model termed Mayo Stratification for Myeloma and Risk-adapted Therapy (mSMART) which divides people into high-risk and standard-risk categories.People with deletion of chromosome 13 or hypodiploidy by conventional cy-

togenetics, t(4;14), t(14;16), t(14;20) or 17p- by molecular genetic studies, or with a high plasma cell labeling index (3% or more) are considered to have high-risk myeloma.

Medical imaging

The diagnostic examination of a person with suspected multiple myeloma typically includes a skeletal survey. This is a series of X-rays of the skull, axial skeleton and proximal long bones. Myeloma activity sometimes appears as "lytic lesions" (with local disappearance of normal bone due to resorption), and on the skull X-ray as "punched-out lesions" (pepper pot skull). Lesions may also be sclerotic which is seen as radiodense. Overall, the radiodensity of myeloma is between −30 and 120 Hounsfield units (HU). Magnetic resonance imaging (MRI) is more sensitive than simple X-ray in the detection of lytic lesions, and may supersede skeletal survey, especially when vertebral disease is suspected. Occasionally a CT scan is performed to measure the size of soft tissue plasmacytomas. Bone scans are typically not of any additional value in the workup of myeloma patients (no new bone formation; lytic lesions not well visualized on bone scan)

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Bleeding Disorders

Bleeding disorders

The abnormal bleeding is come from: Vascular disorders, Thrombocytopenia, Defective platelet function or Defective coagulation. Vascular and platelet disorders tend to be associated with bleeding from mucous membranes and into the skin, whereas in coagulation disorders the bleeding is often into joints or soft tissue.

Vascular disorders

A heterogeneous group of conditions characterized by easy bruising and spontaneous bleeding from the small vessels. It's classified into hereditary causes and acquired causes.

Hereditary vascular abnormality

Giant cavernous haemangioma

These congenital malformations occasionally cause chronic activation of coagulation leading to laboratory features of disseminated intravascular coagulation (DIC) and in some cases thrombocytopenia.

Hereditary haemorrhagic telangiectasia

An autosomal dominant trait transmitted disease. Various genetic defects underlie the disease such as mutations of the endothelial protein, endoglin. There are dilated microvascular swellings which appear during childhood and become more numerous in adult life .Its develop in the skin, mucous membranes and internal organs. Pulmonary, hepatic, splenic and cerebral arteriovenous shunts are seen in a minority of cases and may need local treatment. Recurrent epistaxes are frequent and recurrent gastrointestinal tract haemorrhage may cause chronic iron deficiency anaemia.

Ehlers–Danlos syndromes

There are hereditary collagen abnormalities with purpura resulting from defective platelet adhesion, hyperextensibility of joints and hyperelastic friable skin. Pseudoxanthoma elasticum is associated with arterial haemorrhage and thrombosis.

Acquired vascular disorders

Purpura associated with infections, mainly of bacterial, viral or rickettsial origin.

The Henoch–Schonlein syndrome is usually seen in children and often follows an acute upper respiratory tract infection.

Scurvy. In vitamin C deficiency defective collagen may cause perifollicular petechiae, bruising and mucosal haemorrhage

Steroid purpura

Simple easy bruising, in healthy women, especially those of child-bearing age.

Senile purpura caused by atrophy of the supporting tissues of cutaneous blood vessels is seen mainly on dorsal aspects of the fore-

arms and hands.

Thrombocytopenia

Thrombocytopenia is a condition characterized by abnormally low levels of thrombocytes, (also known as platelets). A normal human platelet count ranges (from 150,000 to 450,000 platelets per microliter of blood). These limits are determined by the 2.5th lower and upper percentile, so values outside this range do not necessarily indicate disease. One common definition of thrombocytopenia requiring emergency treatment is a platelet count below 50,000 per microliter.

Signs and symptoms

Thrombocytopenia usually has no symptoms and is picked up on a routine full blood count (or complete blood count). Some individuals with thrombocytopenia may experience external bleeding such as nosebleeds, and/or bleeding gums. Some women may have heavier or longer periods or breakthrough bleeding. Bruising, particularly purpura in the forearms and petechiae in the feet, legs, and mucous membranes, may be caused by spontaneous bleeding under the skin (1).

Eliciting a full medical history is vital to ensure the low platelet count is not secondary to another disorder. It is also important to ensure that the other blood cell types, such as red blood cells and white blood cells, are not also suppressed. Painless, round and pinpoint (1 to 3 mm in diameter) petechiae usually appear and fade, and sometimes group to form ecchymoses. Larger than petechiae, ecchymoses are purple, blue or yellow-green areas of skin that vary in size and shape. They can occur anywhere on the body.

A person with this disease may also complain of malaise, fatigue and general weakness (with or without accompanying blood loss). Acquired thrombocytopenia may be associated with the use of certain drugs. Inspection typically reveals evidence of bleeding (petechiae or ecchymoses), along with slow, continuous bleeding from any injuries or wounds. Adults may have large, blood-filled bullae in the mouth. If the person's platelet count is between 30,000 and 50,000/mm3, bruising with minor trauma may be expected; if it is between 15,000 and 30,000/mm3, spontaneous bruising will be seen (mostly on the arms and legs(.

Causes

Thrombocytopenia can be inherited or acquired.

Decreased production: Abnormally low platelet production may be caused by:

Dehydration, Vitamin B12 or folic acid deficiency.

Leukemia or myelodysplastic syndrome or aplastic anemia.

Decreased production of thrombopoietin by the liver in liver failure.

Sepsis, systemic viral or bacterial infection.

Leptospirosis.

Hereditary syndromes.

Congenital amegakaryocytic thrombocytopenia.

Thrombocytopenia absent radius syndrome.

Fanconi anemia.

Bernard-Soulier syndrome (associated with large platelets).

May-Hegglin anomaly.

Grey platelet syndrome.

Alport syndrome.

Wiskott–Aldrich syndromeIncreased destruction.

Abnormally high rates of platelet destruction may be due to immune or non-immune conditions, including:

Immune thrombocytopenic purpura

Thrombotic thrombocytopenic purpura

Hemolytic-uremic syndrome

Disseminated intravascular coagulation

Paroxysmal nocturnal hemoglobinuria

Antiphospholipid syndrome

Systemic lupus erythematosus

Post-transfusion purpura

Neonatal alloimmune thrombocytopenia

Hypersplenism

Dengue fever

Gaucher's disease

Zika virusMedication-induced

The following medications can induce thrombocytopenia through direct myelosuppression:

Valproic acid

Methotrexate

Carboplatin

Interferon

Isotretinoin

Panobinostat

H2 blockers and proton-pump inhibitors

Other causes

Lab error, possibly due to the anticoagulant EDTA in CBC specimen tubes.

Snakebite

Niacin toxicity

Lyme disease

Thrombocytapheresis (also called Plateletpheresis)

Diagnosis

Laboratory tests for thrombocytope-

nia might include full blood count, liver enzymes, kidney function, vitamin B12 levels, folic acid levels, erythrocyte sedimentation rate, and peripheral blood smear. If the cause for the low platelet count remains unclear, a bone marrow biopsy is usually recommended to differentiate cases of decreased platelet production from cases of peripheral platelet destruction(2).

Thrombocytopenia in hospitalized alcoholics may be caused by spleen enlargement, folate deficiency, and, most frequently, the direct toxic effect of alcohol on production, survival time, and function of platelets. Platelet count begins to rise after 2 to 5 days' abstinence from alcohol. The condition is generally benign, and clinically significant hemorrhage is rare.

In severe thrombocytopenia, a bone marrow study can determine the number, size and maturity of the megakaryocytes. This information may identify ineffective platelet production as the cause of thrombocytopenia and rule out a malignant disease process at the same time.

Neonatal thrombocytopenia

Thrombocytopenia affects a few percent of newborns, and its prevalence in neonatal intensive care units (NICU) is high. Normally, it is mild and resolves without consequences. Most cases affect preterm birth infants and result from placental insufficiency and/or fetal hypoxia. Other causes, such as alloimmunity, genetics, autoimmunity, and infection, are less frequent (3).

Thrombocytopenia that starts after the first 72 hours since birth is often the result of underlying sepsis or necrotizing enterocolitis (NEC). In the case of infection, PCR tests may be useful for rapid pathogen identification and detection of antibiotic resistance genes. Possible pathogens include viruses (e.g. Cytomegalovirus (CMV), rubella virus, HIV), bacteria (e.g. Staphylococcus sp., Enterococcus sp., Streptococcus agalactiae (GBS), Listeria monocytogenes, Escherichia coli, Haemophilus influenzae, Klebsiella pneumoniae, Pseudomonas aeruginosa, Yersinia enterocolitica), fungi (e.g. Candida sp.), and Toxoplasma gondii. The severity of thrombocytopenia may be correlated with pathogen type; some research indicates that the most severe cases are related to fungal or gram-negative bacterial infection. The pathogen may be transmitted during or before birth, by breast feeding, or during transfusion. Interleukin-11 is being investigated as a drug for managing thrombocytopenia, especially in cases of sepsis or necrotizing enterocolitis (NEC).

Increased destruction of platelets

Autoimmune (idiopathic) thrombocytopenic purpura

Autoimmune (idiopathic) thrombocytopenic purpura (ITP) may be divided into chronic and acute forms.

Chronic idiopathic thrombocytopenic purpura (ITP)

This is a relatively common disorder. The highest incidence has been considered to be in women aged 15–50 years, although some reports suggest an increasing incidence with age. It is the most common cause of thrombocytopenia without anaemia or neutropenia. It is usually idiopathic but may be seen in association with other diseases such as systemic lupus erythematosus (SLE), human immunodeficiency virus (HIV) infection, viral hepatitis, Helicobacter pylori infection, chronic lymphocytic leukaemia (CLL), Hodgkin lymphoma or autoimmune haemolytic anaemia.

Pathogenesis

Platelet autoantibodies, usually IgG, result in the premature removal of platelets from the circulation by macrophages of the reticuloendothelial system, especially the spleen. In many cases, the antibody is directed against the glycoprotein (GP) IIb/IIIa or Ib complex. The normal lifespan of a platelet is 10 days but in ITP this is reduced to a few hours. Total megakaryocyte mass and platelet turnover are increased in parallel to approximately five times normal.

Acute idiopathic thrombocytopenic purpura

This is most common in children. In approximately 75% of patients the episode follows vaccination or an infection such as chickenpox or infectious mononucleosis. Most cases are caused by non-specific immune complex attachments to platelets. Spontaneous remissions are usual but in 5–10% of cases the disease becomes chronic, lasting more than 6 months. Fortunately, morbidity and mortality in acute ITP is very low. The main risk is of cerebral haemorrhage, fortunately rare. Most children do not have any bleeding even with platelet counts $<10 \times 109/L$ but need to avoid trauma such as contact sports.

Thrombotic thrombocytopenic purpura

Thrombotic thrombocytopenic purpura (TTP) is a blood disorder that results in blood clots forming in small blood vessels throughout the body. This results in a low platelet count, low red blood cells due to their breakdown, and often kidneys, heart, and brain dysfunction (4). Symptoms may include large bruises, fever, and weakness, shortness of breath, confusion, and headache. Repeated episodes may occur. Other names Moschcowitz syndrome, idiopathic thrombotic thrombocytopenic purpura.

In about half of cases a trigger is identified, while in the remainder the cause remains unknown. Known triggers include bacterial infections, certain medications, autoimmune diseases such as lupus, and pregnancy. The underlying mechanism typically involves antibodies inhibiting the enzyme ADAMTS13. This results in decreased

break down of large multimers of von Willebrand factor (vWF) into smaller units. Less commonly TTP is inherited from a person's parents, known as Upshaw–Schulman syndrome, such that ADAMTS13 dysfunction is present from birth. Diagnosis is typically based on symptoms and blood tests. It may be supported by measuring activity of or antibodies against ADAMTS13.

Genetics

Thrombotic thrombocytopenic purpura is inherited in an autosomal recessive manner. This condition may also be congenital. Such cases may be caused by mutations in the ADAMTS13 gene. This hereditary form of TTP is called the Upshaw-Schulman syndrome. People with this inherited ADAMTS13 deficiency have a surprisingly mild phenotype, but develop TTP in clinical situations with increased von-Willebrand factor levels, e.g. infection. Reportedly, less than 1% of all TTP cases are due to Upshaw-Schulman syndrome. People with this syndrome generally have 5–10% of normal ADAMTS-13 activity.

Secondary TTP is diagnosed when the person's history mentions one of the known features associated with TTP. It comprises about 40% of all cases of TTP. Predisposing factors are:

Cancer

Bone marrow transplantation

Pregnancy

Medication use:

Antiviral drugs (acyclovir).

Certain chemotherapy medications such as gemcitabine and mitomycin C.

Quinine.

Oxymorphone.

Quetiapine.

Bevacizumab.

Sunitinib.

Platelet aggregation inhibitors (ticlopidine, clopidogrel, and prasugrel).

Immunosuppressants (ciclosporin, mitomycin, tacrolimus/FK506, interferon-α).

Hormone altering drugs (estrogens, contraceptives, hormone replacement therapy).

HIV-1 infection

The mechanism of secondary TTP is poorly understood, as ADAMTS13 activity is generally not as depressed as in idiopathic TTP, and inhibitors cannot be detected. Probable etiology may involve, at least in some cases, endothelial damage, although the formation of thrombi resulting in vessel occlusion may not be essential in the pathogenesis of secondary TTP. These factors may also be considered a form of secondary aHUS; people presenting with these features are, therefore, potential candidates for anticomplement therapy.

Signs and symptoms

The signs and symptoms of TTP may at first be subtle and nonspecific. Many people experience an influenza-like or diarrheal illness before developing TTP. Neurological symptoms are very common and vary greatly in severity. Frequently reported symptoms include feeling very tired, confusion, and headaches (5). Seizures and symptoms similar to those of a stroke can also be seen. Other symptoms include, but are not limited to jaundice or paleness of the skin, a fast heart rate or shortness of breath, or pinpoint-sized purple or reddish dots on the skin known as petechiae.

As TTP progresses, blood clots form within small blood vessels (microvasculature), and platelets (clotting cells) are consumed. As a result, bruising, and rarely bleeding can occur. The bruising often takes the form of purpura, while the most common site of bleeding, if it occurs, is from the nose or gums. Larger bruises (ecchymoses) may also develop.

The classic presentation of TTP, which occurs in less than 10% of people, includes five medical signs. These are:

Fever

Changes in mental status

Thrombocytopenia

Reduced kidney function

Haemolytic anaemia (microangiopathic hemolytic anemia).

Causes

TTP, as with other microangiopathic hemolytic anemias (MAHAs), is caused by spontaneous aggregation of platelets and activation of coagulation in the small blood vessels. Platelets are consumed in the aggregation process and bind vWF. These platelet-vWF complexes form small blood clots which circulate in the blood vessels and cause shearing of red blood cells, resulting in their rupture and formation of schistocytes.

The two best understood causes of TTP are due to autoimmunity and an inherited deficiency of ADAMTS13 (known as the Upshaw-Schülman syndrome). The majority of the remaining cases are secondary to some other factor.

Disseminated intravascular coagulation

In this disorder thrombocytopenia may result from a high rate of platelet destruction due to increased levels of consumption.

Increased splenic pooling

The major factor responsible for thrombocytopenia in splenomegaly is platelet 'pooling' by the spleen. In splenomegaly, up to 90% of platelets may be sequestered in the spleen.

Massive transfusion syndrome

Patients transfused with massive amounts

of stored blood, such as more than 10 units over a 24-hour period, frequently show abnormal clotting and thrombocytopenia.

Disorders of platelet function

Hereditary disorders

Bernard-Soulier syndrome is a defect or deficiency in GPIb. GPIb, the receptor for vWF, can be defective and lead to lack of primary clot formation (primary hemostasis) and increased bleeding tendency. This is an autosomal recessive inherited disorder.

Glanzmann thrombasthenia is extremely rare. It is characterized by a defect in GPIIb/IIIa fibrinogen receptor complex. When GPIIb/IIIa receptor is dysfunctional, fibrinogen cannot cross-link platelets, which inhibits primary hemostasis. This is an autosomal recessive inherited disorder.

Storage pool diseases

A mutation in a growth factor independent gene (GFI1B) underlies the grey-platelet syndrome. The platelets are larger than normal, with virtual absence of alpha granules. In the more common delta-storage pool disease, there is deficiency of dense granules. Platelet-dependent haemostasis is abnormal in the much more common von Willebrand disease because of an inherited defect in VWF.

Acquired disorders

Antiplatelet drugs such as Aspirin (the most common), Dipyridamole (inhibits platelet aggregation by blocking reuptake of adenosine) and the Clopidogrel and prasugrel which inhibit binding of ADP to its platelet receptor.

Diagnosis of platelet disorders

Patients with suspected platelet or blood vessel abnormalities should initially have a blood count and blood film examination. Bone marrow examination is often needed in thrombocytopenic patients to determine whether or not there is a failure of platelet production. The marrow may also reveal one of the conditions associated with defective production. In children and young adults with isolated thrombocytopenia, the marrow test is often not performed. In the elderly, marrow examination is needed particularly to exclude myelodysplasia. In patients with thrombocytopenia, normal haemoglobin and white cell counts, a negative drug history, normal or excessive numbers of marrow megakaryocytes and no other marrow abnormality or splenomegaly, ITP is the usual diagnosis.

Coagulation disorders

Coagulation factor disorders devided to hereditary and acquired. The best-known coagulation factor disorders are the hemophilias. The three main forms are hemophilia A (factor VIII deficiency), hemophilia B

(factor IX deficiency or "Christmas disease") and hemophilia C (factor XI deficiency, mild bleeding tendency). Hemophilia A and B are X-linked recessive disorders, whereas Hemophilia C is a much more rare autosomal recessive disorder most commonly seen in Ashkenazi Jews.

Hereditary coagulation disorders

Haemophilia

Haemophilia is a mostly inherited genetic disorder that impairs the body's ability to make blood clots, a process needed to stop bleeding. This results in people bleeding longer after an injury, easy bruising, and an increased risk of bleeding inside joints or the brain (6). Those with a mild case of the disease may have symptoms only after an accident or during surgery. Bleeding into a joint can result in permanent damage while bleeding in the brain can result in long term headaches, seizures, or a decreased level of consciousness.

There are two main types of haemophilia: haemophilia A, which occurs due to not enough clotting factor VIII, and haemophilia B, which occurs due to not enough clotting factor IX. They are typically inherited from one's parents through an X chromosome with a nonfunctional gene. Rarely a new mutation may occur during early development or haemophilia may develop later in life due to antibodies forming against a clotting factor. Other types include haemophilia C, which occurs due to not enough factor XI, and parahaemophilia, which occurs due to not enough factor V. Acquired haemophilia is associated with cancers, autoimmune disorders, and pregnancy. Diagnosis is by testing the blood for its ability to clot and its levels of clotting factors.

Prevention may occur by removing an egg, fertilizing it, and testing the embryo before transferring it to the uterus. Treatment is by replacing the missing blood clotting factors. This may be done on a regular basis or during bleeding episodes. Replacement may take place at home or in hospital. The clotting factors are made either from human blood or by recombinant methods. Up to 20% of people develop antibodies to the clotting factors which makes treatment more difficult. The medication desmopressin may be used in those with mild haemophilia A. Studies of gene therapy are in early human trials.

Haemophilia A affects about 1 in 5,000–10,000, while haemophilia B affects about 1 in 40,000, males at birth. As haemophilia A and B are both X-linked recessive disorders, females are rarely severely affected. Some females with a nonfunctional gene on one of the X chromosomes may be mildly symptomatic. Haemophilia C occurs equally in both sexes and is mostly found in Ashkenazi Jews. In the 1800s haemophilia B was common within the royal families of Europe. The difference between haemophilia A and

B was determined in 1952. The word is from the Greek haima αἷ μα meaning blood and philia φιλία meaning love.

Signs and symptoms

Characteristic symptoms vary with severity. In general symptoms are internal or external bleeding episodes, which are called "bleeds". People with more severe haemophilia suffer more severe and more frequent bleeds, while people with mild haemophilia usually suffer more minor symptoms except after surgery or serious trauma. In cases of moderate haemophilia symptoms are variable which manifest along a spectrum between severe and mild forms.

In both haemophilia A and B, there is spontaneous bleeding but a normal bleeding time, normal prothrombin time, normal thrombin time, but prolonged partial thromboplastin time. Internal bleeding is common in people with severe haemophilia and some individuals with moderate haemophilia. The most characteristic type of internal bleed is a joint bleed where blood enters into the joint spaces (7). This is most common with severe haemophiliacs and can occur spontaneously (without evident trauma). If not treated promptly, joint bleeds can lead to permanent joint damage and disfigurement. Bleeding into soft tissues such as muscles and subcutaneous tissues is less severe but can lead to damage and requires treatment.

Children with mild to moderate haemophilia may not have any signs or symptoms at birth especially if they do not undergo circumcision. Their first symptoms are often frequent and large bruises and haematomas from frequent bumps and falls as they learn to walk. Swelling and bruising from bleeding in the joints, soft tissue, and muscles may also occur. Children with mild haemophilia may not have noticeable symptoms for many years. Often, the first sign in very mild haemophiliacs is heavy bleeding from a dental procedure, an accident, or surgery. Females who are carriers usually have enough clotting factors from their one normal gene to prevent serious bleeding problems, though some may present as mild haemophiliacs.

Complications

Severe complications are much more common in cases of severe and moderate haemophilia. Complications may arise from the disease itself or from its treatment:

Deep internal bleeding, e.g. deep-muscle bleeding, leading to swelling, numbness or pain of a limb.

Joint damage from haemarthrosis (haemophilic arthropathy), potentially with severe pain, disfigurement, and even destruction of the joint and development of debilitating arthritis.

Transfusion transmitted infection from blood transfusions that are given as treatment.

Adverse reactions to clotting factor treatment, including the development of an

immune inhibitor which renders factor replacement less effective.

Intracranial haemorrhage is a serious medical emergency caused by the buildup of pressure inside the skull. It can cause disorientation, nausea, loss of consciousness, brain damage, and death.

Haemophilic arthropathy is characterized by chronic proliferative synovitis and cartilage destruction. If an intra-articular bleed is not drained early, it may cause apoptosis of chondrocytes and affect the synthesis of proteoglycans. The hypertrophied and fragile synovial lining while attempting to eliminate excessive blood may be more likely to easily rebleed, leading to a vicious cycle of hemarthrosis-synovitis-hemarthrosis. In addition, iron deposition in the synovium may induce an inflammatory response activating the immune system and stimulating angiogenesis, resulting in cartilage and bone destruction.

Genetics

X-linked recessive inheritance. Females possess two X-chromosomes, males have one X and one Y-chromosome. Since the mutations causing the disease are X-linked recessive, a female carrying the defect on one of her X-chromosomes may not be affected by it, as the equivalent allele on her other chromosome should express itself to produce the necessary clotting factors, due to X inactivation. However, the Y-chromosome in the male has no gene for factors VIII or IX. If the genes responsible for production of factor VIII or factor IX present on a male's X-chromosome are deficient there is no equivalent on the Y-chromosome to cancel it out, so the deficient gene is not masked and the disorder will develop.

Since a male receives his single X-chromosome from his mother, the son of a healthy female silently carrying the deficient gene will have a 50% chance of inheriting that gene from her and with it the disease; and if his mother is affected with haemophilia, he will have a 100% chance of being a haemophiliac. In contrast, for a female to inherit the disease, she must receive two deficient X-chromosomes, one from her mother and the other from her father (who must therefore be a haemophiliac himself). Hence, haemophilia is expressed far more commonly among males than females, while double-X females are far more likely to be silent carriers, survive childhood and to submit each of her genetic children to an at least 50% risk of receiving the deficient gene. However, it is possible for female carriers to become mild haemophiliacs due to lyonisation (inactivation) of the X-chromosomes. Haemophiliac daughters are more common than they once were, as improved treatments for the disease have allowed more haemophiliac males to survive to adulthood and become parents. Adult females may experience menorrhagia (heavy periods) due to the bleeding tendency. The pattern of inheritance is criss-cross type. This type of pattern is also seen in colour blindness.

A mother who is a carrier has a 50% chance of passing the faulty X-chromosome to her daughter, while an affected father will always pass on the affected gene to his daughters. A son cannot inherit the defective gene from his father. This is a recessive trait and can be passed on if cases are more severe with carrier. Genetic testing and genetic counselling is recommended for families with haemophilia. Prenatal testing, such as amniocentesis, is available to pregnant women who may be carriers of the condition.

As with all genetic disorders, it is of course also possible for a human to acquire it spontaneously through mutation, rather than inheriting it, because of a new mutation in one of their parents' gametes. Spontaneous mutations account for about 33% of all cases of haemophilia A. About 30% of cases of haemophilia B are the result of a spontaneous gene mutation.

If a female gives birth to a haemophiliac son, either the female is a carrier for the blood disorder or the haemophilia was the result of a spontaneous mutation. Until modern direct DNA testing, however, it was impossible to determine if a female with only healthy children was a carrier or not. Generally, the more healthy sons she bore, the higher the probability that she was not a carrier.

If a male is afflicted with the disease and has children with a female who is not a carrier, his daughters will be carriers of haemophilia. His sons, however, will not be affected with the disease. The disease is X-linked and the father cannot pass haemophilia through the Y-chromosome. Males with the disorder are then no more likely to pass on the gene to their children than carrier females, though all daughters they sire will be carriers and all sons they father will not have haemophilia (unless the mother is a carrier).

Severity

There are numerous different mutations which cause each type of haemophilia. Due to differences in changes to the genes involved, people with haemophilia often have some level of active clotting factor. Individuals with less than 1% active factor are classified as having severe haemophilia, those with 1-5% active factor have moderate haemophilia, and those with mild haemophilia have between 5-40% of normal levels of active clotting factor.

Diagnosis

Haemophilia can be diagnosed before, during or after birth if there is a family history of the condition. Several options are available to parents. If there is no family history of haemophilia, it is usually only diagnosed when a child begins to walk or crawl. They may experience joint bleeds or easy bruising (8).

Mild haemophilia may only be discovered later, usually after an injury or a dental or surgical procedure.

Before pregnancy

Genetic testing and counselling are available to help determine the risk of passing the condition onto a child. This may involve testing a sample of tissue or blood to look for signs of the genetic mutation that causes haemophilia.

During pregnancy

A pregnant woman with a history of haemophilia in her family can test for the haemophilia gene. Such tests include:

chorionic villus sampling (CVS) – a small sample of the placenta is removed from the womb and tested for the haemophilia gene, usually during weeks 11-14 of pregnancy

amniocentesis – a sample of amniotic fluid is taken for testing, usually during weeks 15-20 of pregnancy

There's a small risk of these procedures causing problems such as miscarriage or premature labour, so the woman may discuss this with the doctor in charge of her care.

After birth

If haemophilia is suspected after a child has been born, a blood test can usually confirm the diagnosis. Blood from the umbilical cord can be tested at birth if there's a family history of haemophilia. A blood test will also be able to identify whether a child has haemophilia A or B, and how severe it is (8).

Classification

There are several types of haemophilia: haemophilia A, haemophilia B, haemophilia C, parahaemophilia, and acquired haemophilia A.

Haemophilia A, is a recessive X-linked genetic disorder resulting in a deficiency of functional clotting Factor VIII. Haemophilia B, is also a recessive X-linked genetic disorder involving a lack of functional clotting Factor IX. Haemophilia C, is an autosomal genetic disorder involving a lack of functional clotting Factor XI. Haemophilia C is not completely recessive, as heterozygous individuals also show increased bleeding (9).

The type of haemophilia known as parahaemophilia is a mild and rare form and is due to a deficiency in factor V. This type can be inherited or acquired.

A non-genetic form of haemophilia is caused by autoantibodies against factor VIII and so is known as acquired haemophilia A. Acquired haemophilia can be associated with cancers, autoimmune disorders and following childbirth.

Management

There is no long-term cure. Treatment and prevention of bleeding episodes is done primarily by replacing the missing blood clotting factors.

Clottingfactors

Commercially produced factor concentrates

such as "Advate", a recombinant Factor VIII, come as a white powder in a vial which must be mixed with sterile water prior to intravenous injection.

Clotting factors are usually not needed in mild haemophilia. In moderate haemophilia clotting factors are typically only needed when bleeding occurs or to prevent bleeding with certain events. In severe haemophilia preventive use is often recommended two or three times a week and may continue for life. Rapid treatment of bleeding episodes decreases damage to the body.

Factor VIII is used in haemophilia A and factor IX in haemophilia B. Factor replacement can be either isolated from human blood serum, recombinant, or a combination of the two. Some people develop antibodies (inhibitors) against the replacement factors given to them, so the amount of the factor has to be increased or non-human replacement products must be given, such as porcine factor VIII.

If a person becomes refractory to replacement coagulation factor as a result of high levels of circulating inhibitors, this may be partially overcome with recombinant human factor VIII.

In early 2008, the US Food and Drug Administration (FDA) approved anti-haemophilic factor genetically engineered from the genes of Chinese hamster ovary cells. Since 1993 recombinant factor products (which are typically cultured in Chinese hamster ovary (CHO) tissue culture cells and involve little, if any human plasma products) have been available and have been widely used in wealthier western countries. While recombinant clotting factor products offer higher purity and safety, they are, like concentrate, extremely expensive, and not generally available in the developing world. In many cases, factor products of any sort are difficult to obtain in developing countries.

Clotting factors are either given preventively or on-demand. Preventive use involves the infusion of clotting factor on a regular schedule in order to keep clotting levels sufficiently high to prevent spontaneous bleeding episodes. On-demand (or episodic) treatment involves treating bleeding episodes once they arise. In 2007, a trial comparing on-demand treatment of boys (< 30 months) with haemophilia A with prophylactic treatment (infusions of 25 IU/kg body weight of Factor VIII every other day) in respect to its effect on the prevention of joint-diseases. When the boys reached 6 years of age, 93% of those in the prophylaxis group and 55% of those in the episodic-therapy group had a normal index joint-structure on MRI. Preventative treatment, however, resulted in average costs of $300,000 per year. The author of an editorial published in the same issue of the NEJM supports the idea that prophylactic treatment not only is more effective than on demand treatment but also suggests that starting after the first serious joint-related haemorrhage may be more cost effective than waiting until the fixed age to begin.

Most haemophiliacs in third world countries have limited or no access to commercial blood clotting factor products.

Other

Desmopressin (DDAVP) may be used in those with mild haemophilia A. Tranexamic acid or epsilon aminocaproic acid may be given along with clotting factors to prevent breakdown of clots.

Pain medicines, steroids, and physical therapy may be used to reduce pain and swelling in an affected joint. In those with severe hemophilia A already receiving FVIII, emicizumab may provide some benefit.

Contraindications

Anticoagulants such as heparin and warfarin are contraindicated for people with haemophilia as these can aggravate clotting difficulties. Also contraindicated are those drugs which have "blood thinning" side effects. For instance, medicines which contain aspirin, ibuprofen, or naproxen sodium should not be taken because they are well known to have the side effect of prolonged bleeding.

Also contraindicated are activities with a high likelihood of trauma, such as motorcycling and skateboarding. Popular sports with very high rates of physical contact and injuries such as American football, hockey, boxing, wrestling, and rugby should be avoided by people with haemophilia. Other active sports like soccer, baseball, and basketball also have a high rate of injuries, but have overall less contact and should be undertaken cautiously and only in consultation with a doctor.

Prognosis

Like most aspects of the disorder, life expectancy varies with severity and adequate treatment. People with severe haemophilia who don't receive adequate, modern treatment have greatly shortened lifespans and often do not reach maturity. Prior to the 1960s when effective treatment became available, average life expectancy was only 11 years. By the 1980s the life span of the average haemophiliac receiving appropriate treatment was 50–60 years. Today with appropriate treatment, males with haemophilia typically have a near normal quality of life with an average lifespan approximately 10 years shorter than an unaffected male.

Since the 1980s the primary leading cause of death of people with severe haemophilia has shifted from haemorrhage to HIV/AIDS acquired through treatment with contaminated blood products. The second leading cause of death related to severe haemophilia complications is intracranial haemorrhage which today accounts for one third of all deaths of people with haemophilia. Two other major causes of death include hepatitis infections causing cirrhosis and obstruction of air or blood flow due to soft tissue haemorrhage.

Epidemiology

Haemophilia is rare, with only about 1 in-

stance in every 10,000 births (or 1 in 5,000 male births) for haemophilia A and 1 in 50,000 births for haemophilia B. About 18,000 people in the United States have haemophilia. Each year in the US, about 400 babies are born with the disorder. Haemophilia usually occurs in males and less often in females. It is estimated that about 2,500 Canadians have haemophilia A, and about 500 Canadians have haemophilia B.

Hereditary deficiency of other coagulation factors

All these disorders (deficiency of fibrinogen, prothrombin, factors V, VII, combined V and VIII, factors X, XI, XIII or mutation of thrombomodulin) are rare. In all the inheritance is autosomal recessive except for factor XI deficiency where there is variable penetrance. Recombinant factor VII is available for therapy. Factor XI deficiency is seen mainly in Ashkenazi Jews and occurs in either sex. The bleeding risk shows incomplete correlation to severity of the deficiency. Bleeding only occurs after trauma such as surgery. Treatment is with fibrinolytic inhibitor, factor XI concentrate or fresh frozen plasma. Factor XIII deficiency produces a severe bleeding tendency, characteristically with umbilical stump bleeding. Plasma concentrates and recombinant preparation of factor XIII are available.

Acquired coagulation disorders

The acquired coagulation disorders are more common than the inherited disorders.

Vitamin K deficiency

Fat-soluble vitamin K is obtained from green vegetables and bacterial synthesis in the gut. Deficiency may present in the newborn (haemorrhagic disease of the newborn) or in later life. Deficiency of vitamin K is caused by an inadequate diet, malabsorption or inhibition of vitamin K by vitamin K antagonist drugs such as warfarin. Warfarin is associated with a decrease in the functional activity of factors II, VII, IX and X and proteins C and S, but immunological methods show normal levels of these factors. The non-functional proteins are called PIVKA (proteins formed in vitamin K absence). Conversion of PIVKA factors to their biologically active forms is a post-translational event involving carboxylation of glutamic acid residues in the N-terminal region. Gamma-carboxylated glutamic acid binds calcium ions, inducing a reversible shape change in the N-termini of vitamin K dependent

Proteins. This exposes hydrophobic residues which bind to phospholipid. In the process of carboxylation, vitamin K is converted to vitamin K epoxide, which is cycled back to the reduced form by a reductase (VKORC-1). Warfarin interferes with the action of vitamin K epoxide reductase leading to a functional vitamin K deficiency.

Haemorrhagic disease of the newborn

Vitamin K-dependent factors are low at birth and fall further in breast-fed infants in the first few days of life. Liver cell immaturity, lack of gut bacterial synthesis of the vitamin and low quantities in breast milk may all contribute to a deficiency which causes haemorrhage, usually on the second to fourth day of life, but occasionally during the first 2 months.

Diagnosis

The PT and APTT are both abnormal. The platelet count and fibrinogen are normal with absent fibrin degradation products.

Treatment

1. Prophylaxis. For many years vitamin K has been givento all newborn babies as a single intramuscular injection of 1 mg. This remains the most appropriate and safest treatment. Following epidemiological evidence suggesting a possible link between intramuscular vitamin K and an increased risk of childhood tumours (which has not been substantiated), some centres recommended an oral regimen but this has never been subjected to randomizedcontrolled trial.

2. In bleeding infants: vitamin K 1 mg intramuscularly is given every 6 hours with, initially, prothrombin complex concentrate if haemorrhage is severe.

Disseminated intravascular coagulation

Widespread inappropriate intravascular deposition of fibrin with consumption of coagulation factors and platelets occurs as a consequence of many disorders that release procoagulant material into the circulation or cause widespread endothelial damage or platelet aggregation. It may be associated with a fulminant haemorrhagicor thrombotic syndrome with organ dysfunction or run a lesssevere and more chronic course. The main clinical presentationis with bleeding but 5–10% of patients manifest thromboticlesions (e.g. with gangrene of limbs).

Pathogenesis

The key event underlying DIC is increased activity of thrombin in the circulation that overwhelms its normal rate of removal by natural anticoagulants. This can come from tissue factor (TF) release into the circulation from damaged tissues present on tumour cells or from up-regulation of TF on circulating monocytes or endothelial cells in response to proinflammatory cytokines (e.g. interleukin-1, tumour necrosis factor, endotoxin).

1. DIC may be triggered by the entry of procoagulant materialinto the circulation in the following situations: severe trauma, amniotic fluid embolism, premature separation of the placenta, widespread mucin-secreting adenocarcino mas, acute promyelocytic leukaemia, liver disease, severe falciparum ma-

laria, haemolytic transfusion reaction and some snake bites.

2. DIC may also be initiated by widespread endothelial damage and collagen exposure (e.g. endotoxaemia, Gram-negative and meningococcal septicaemia, and septic abortion), certain virus infections and severe burns or hypothermia. Proinflammatory cytokines and activation of monocytes by bacteria up-regulate tissue factor as well as releasing microparticlesexpressing tissue factor into the circulation.

In addition to its role in the deposition of fibrin in the microcirculation, intravascular thrombin formation produces large amounts of circulating fibrin monomers, which form complexes with fibrinogen and interfere with fibrin polymerization, thus contributing to the coagulation defect. Intense fibrinolysis is stimulated by thrombi on vascular walls and the release of split products interferes with fibrin polymerization, thus contributing to the coagulation defect. The combined action of thrombin and plasmin causes depletion of fibrinogen and all coagulation factors. Intravascular thrombin also causes widespread platelet aggregation in the vessels. The bleeding problems which may be a feature of DIC are compounded by thrombocytopenia caused by consumption of platelets.

Clinical features

These are usually dominated by bleeding, particularly from venepuncture sites or wounds. There may be generalized bleeding in the gastrointestinal tract, the oropharynx, into the lungs, urogenital tract and in obstetric cases, vaginal bleeding may be particularly severe. Less frequently, microthrombi may cause skin lesions, renal failure, gangrene of the fingers or toes or cerebral ischaemia. Some patients may develop subacute or chronic DIC, especially with mucin-secreting adenocarcinoma.

Laboratory findings

In many acute syndromes the blood may fail to clot because of gross fibrinogen deficiency.

Tests of haemostasis

1. The platelet count is low.

2. Fibrinogen concentration is low.

3. The thrombin time is prolonged.

4. High levels of fibrin degradation products such as D-dimers are found in serum and urine.

5. The PT and APTT are prolonged in the acute syndromes.

Blood film examination

In many patients there is a haemolytic anaemia ('microangiopathic') and the red cells show prominent fragmentation because of damage caused when passing through fibrin strands in small vessels.

Treatment

Treatment of the underlying cause is most important. The management of patients who are bleeding differs from that of patients with thrombotic problems.

Supportive therapy with fresh frozen plasma and platelet concentrates is indicated in patients with dangerous or extensive bleeding. Cryoprecipitate or fibrinogen concentrates provide more concentrated fibrinogen; red cell transfusions may be required.

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I be more than happy to get you opinions and questions , feel free to contact me:

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Development in science depends on the transmission of information on the level of the individual and more importantly the level of the generation. Transfer of information between individuals increases the strength and guarantees continuity, and it also gives us a lot of time to advance in science.

Writing information and facts in books gives generations a lot of time, so they will start from where the ancestors ended.

So, reader, that what you have of knowledge is a message, you must preserve it and transfer it to your next generation, and not to let it be lost with you, you are mortal, but your knowledge is still.

Author